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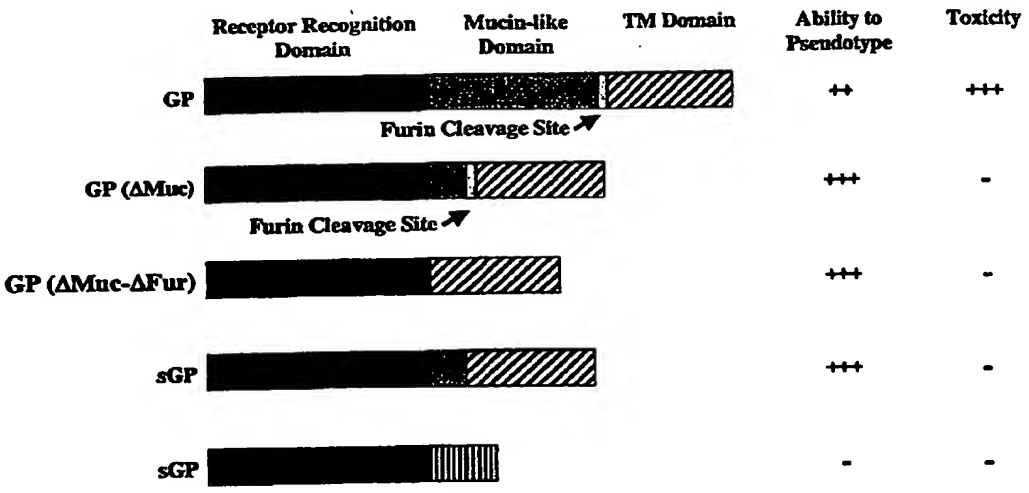

(57) Abstract

The present invention provides compositions and methods for targeting gene transfer vectors to certain cell types by pseudotyping with a transmembrane form of viral glycoprotein, such as that from Ebola virus. The methods comprise the step of administering to a cell population a gene to be transferred operatively linked to an appropriate transfer vehicle, wherein the transfer vehicle is associated with a transmembrane form of viral glycoprotein.

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TARGETING GENE TRANSFER VECTORS TO CERTAIN CELL TYPES BY PSEUDOTYPING WITH VIRAL GLYCOPROTEIN

FIELD OF THE INVENTION

The present invention relates generally to compositions and methods for selective gene transfer, and in particular, to methods for targeting genes to certain cell types, comprising introducing to a cell population the gene to be transferred operatively-linked to an appropriate transfer vehicle, wherein the transfer vehicle is associated with a transmembrane form of viral glycoprotein.

BACKGROUND OF THE INVENTION

Ebola virus has been identified as the cause of several highly lethal outbreaks of hemorrhagic fever. Infection begins typically with flu-like symptoms which often progress rapidly to fatal complications of hemorrhage, fever, and hypotensive shock. Bowen, E.T.W. et al., *Lancet* 1:571 (1977); Centers for Disease Control, *M.M.W.R.* 44:381 (1995); Le Guenno, B. et al., *Lancet* 345:1271 (1995); Peters, C.J. et al., *Fields Virology*, B.N. Fields, D.M. Knipe and P.M. Howley, Eds. (Lippincott-Raven, Philadelphia) p. 1161 (1996). The negative-stranded genome of Ebola virus contains seven structural and regulatory proteins (Sanchez, A. et al., *Virus Res.* 29:215 (1993)), but despite its relative simplicity, the molecular basis for Ebola virus pathogenicity is unknown. Among the viral gene products, the glycoprotein is found in two forms: a secreted form, 50-70 kD (Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996)), synthesized at high levels early in the course of infection, and an alternative transmembrane form, which arises from RNA editing to encode a 120-150 kD glycoprotein that is incorporated into the virion. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996); Volchkov, V.E. et al., *Virology* 214:421 (1995). The first 295 amino acids (aa) of both proteins are identical in the Zaire strain, while sGP contains an additional 69 and GP another 381 COOH- terminal aa residues. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996). The specific cellular targets of these related gene products and their roles in the pathogenesis of Ebola infection have not been characterized.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for targeting gene transfer vectors to certain cell types by pseudotyping with a transmembrane form of viral glycoprotein. In one embodiment, the methods of the invention comprise the

step of administering to a cell population a gene to be transferred operatively-linked to an appropriate transfer vehicle, wherein the transfer vehicle is associated with a transmembrane form of Ebola glycoprotein. In this embodiment, the gene will be targeted to cell types naturally infected with Ebola such as endothelial cells, monocytes and hepatocytes.

Genetic constructs for selective gene transfer into certain cell types are also provided. The genetic constructs of the present invention comprise a gene to be transferred operatively-linked to an appropriate transfer vehicle or carrier, wherein the transfer vehicle or carrier is associated with a transmembrane form of viral glycoprotein. In one embodiment, the transmembrane form of Ebola glycoprotein is expressed on the surface of a virus-based gene-targeting vector, *e.g.*, lentiviral or retroviral vector. In another embodiment, an expressed or synthesized transmembrane glycoprotein is chemically derivatized to a non-biologic gene targeting vehicle.

Additional objects, advantages, and features of the present invention will become apparent from the following description and appended claims, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and subjoined claims and by referencing the following drawings.

Figures 1A-1C show the binding of sGP to neutrophils;

Figures 2A-2D show the infection of different cell types by a GP-pseudotyped vector of the present invention;

Figures 3A-3F show the dependence of sGP binding on CD16b and correlation of binding with neutrophil activation;

Figures 4A-4B show the effect of sGP on neutrophil function;

Figures 5A-5C show the infection rate of cells with a GP-pseudotyped retroviral vector of the present invention;

Figure 6 is a schematic of the plasmid pVR 1012-GP(IC) (Ivory Coast strain of GP, see SEQ ID NO: 1);

Figure 7 is a schematic of the plasmid pVR 1012-GP(S) (Sudan strain of GP, see SEQ ID NO: 2);

Figure 8 is a schematic of the plasmid pVR 1012-GP(Z) (Zaire strain of GP, see SEQ ID NO: 3);

Figure 9 is a schematic of the plasmid pVR 1012-sGP(Z) (Zaire strain of sGP, see SEQ ID NO: 4); and

Figure 10 is a summary of the characterization of GP and sGP derivatives for their ability to pseudotype to induce cytotoxicity in producer cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides genetic constructs and methods for targeting gene transfer vectors to certain cell types by pseudotyping with a transmembrane form of viral glycoprotein. The methods for selective gene transfer of the present invention comprise the step of administering to a cell population a genetic construct of the present invention so that the gene is transferred and expressed in certain cell types present in the cell population. Administration to the cell population may be *ex vivo* or *in vivo*.

The genetic constructs of the present invention comprise a gene to be transferred operatively-linked to an appropriate transfer vehicle or carrier, wherein the transfer vehicle or carrier is associated with a transmembrane form of viral glycoprotein. In one embodiment, the transmembrane form of Ebola glycoprotein is associated with the vehicle or carrier. The gene to be transferred will thus be targeted to cell types naturally infected with Ebola virus including endothelial cells, hepatocytes, monocytes and related cell types such as dendritic cells. The transmembrane form of Ebola glycoprotein may be chosen from, without limitation, the Ivory Coast strain (SEQ ID NO: 1), Sudan strain (SEQ ID NO: 2), the Zaire strain (SEQ ID NO: 3) and/or the Reston strain. It will be appreciated that in other embodiments of the present invention, other hemorrhagic fever virus glycoproteins, in particular transmembrane glycoproteins, may be employed and will target those cell types naturally infected by the virus. Examples of hemorrhagic viruses include dengue virus, Yellow Fever virus (*flaviviridae*); Lassa, Junin and Machupo (*arenaviridae*); Rift Valley, Congo-Crimean and Hantaan (*bunyaviridae*); and Marburg (*filoviridae*). It will also be appreciated that derivatives of the transmembrane glycoprotein which retain the capability of targeting specific cell types, may also be employed, for example, the transmembrane glycoproteins may be mutated, *e.g.*, toxic regions may be removed to improve producer cell viability (see Figure 10).

The transmembrane glycoprotein may be expressed on the surface of a virus-based gene-targeting vector, *e.g.*, lentiviral, retroviral, replication-deficient retroviral, adenoviral or adeno-associated viral vector. The transmembrane glycoprotein may

also be expressed or synthesized and chemically derivatized to a non-biologic gene targeting vehicle, *e.g.*, liposome or DNA-protein complex.

The term "operatively-linked" as used herein refers to functional linkage between a nucleic acid expression control sequence (such as a promoter) and a second nucleic acid sequence (*i.e.*, gene), wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence. Expression control sequences are known to those skilled in the art (see, *e.g.*, Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990)). "Associated with" as used herein refers to the transmembrane form of viral glycoprotein being in contact or linkage with the transfer vehicle or carrier in such a way as to direct the transfer vehicle or carrier to certain cell types. The terms "transfer vehicle" and "carrier" refer to any type of structure which is capable of delivering the gene of interest to a target cell.

Many transfer vehicles or carriers are known in the art. For example, various viruses that are capable of infecting cells can be recombinantly manipulated to carry the gene of interest without affecting their infectivity. As used herein, the terms "infect" and "infectivity" refer only to the ability of a virus to transfer genetic material to a target cell. Those terms do not mean that the virus is capable of replication in the target cell. In fact, it is preferable that such viruses are replication defective so that target cells do not suffer the effects of viral replication.

In one embodiment, the virus employed is a replication defective retroviruses. When these replication defective retroviruses are employed, their genomes can be packaged by a helper virus in accordance with well-known techniques. Suitable retroviruses include PLJ, pZip, pWe and pEM, each of which is well known in the art. Suitable helper viruses for packaging genomes include ψCrip, ψCre, ψ2, ψAm and adeno-associated viruses.

In another embodiment, lentiviral vectors are employed. Surprisingly, the inventors of the present invention were successful in pseudotyping lentiviral vectors (HIV) with the transmembrane glycoprotein from Ebola. Feline immunodeficiency virus, bovine immunodeficiency virus, simian immunodeficiency virus and EAIV, may also be employed as the carrier in the compositions and methods of the present invention.

Gene delivery systems other than viruses may also be employed. For example, the gene to be transferred may be packaged in a liposome which is chemically derivatized to the transmembrane glycoprotein. To form these liposomes,

one mixes the DNA of an expression vector which expresses the gene to be transferred with lipid, such as *N*-[1-(2,3,-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA) in a suitable buffer, such as Hepes buffered saline. This causes the spontaneous formation of lipid-DNA complexes (liposomes). Felgner, P.L. et al., *PNAS (USA)* 84:7413-7417 (1987).

Another gene delivery system that may be utilized in this invention is DNA-protein complexes. The formation of DNA-protein complexes is described in United States Patent No. 5,166,320, the disclosure of which is herein incorporated by reference.

It will be appreciated that any gene may be employed in the compositions and methods of the present invention. For example, and without limitation, in the treatment of cancer, death inducing genes, including genes coding for cytostatic or cytotoxic proteins, *e.g.*, HSV tk, and genes encoding cyclin dependent kinase inhibitors, p21, p27, cytosine deaminase, and fas ligand, may all be employed. In another example, for the treatment of cardiovascular or ischemic vascular disease, genes encoding angiogenic factors such as VEGF basic or acidic FGF's (FGF 1-5) may be employed. In yet another example, in the treatment of vasospasm, the gene encoding NO synthase or heme oxygenase, may be employed. In a further example, monocytes and dendritic cells may be targeted with genes encoding immunogens for cell-targeted immunization.

In one embodiment, the methods of targeting gene transfer vectors to certain cell types involve administering to a cell population *ex vivo*, a construct of the present invention and introducing the transfected cells into a subject. In an alternative embodiment, the methods of the present invention comprise administering to an *in vivo* cell population a construct of the present invention. Administration can be by any of the routes normally used for *in vivo* gene therapy such as direct delivery to cells via a gene gun, and other known techniques. The constructs are thus administered in any suitable manner, preferably with pharmaceutically acceptable carriers. The constructs can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. The preferred method of administration will often be intravenous.

To practice an *ex vivo* method of the present invention, a source of cells is obtained. The cells are optionally selected from *in vitro* cells, such as those derived from cell culture and *ex vivo* cells, such as those derived from a subject. The term "subject" is intended to include living organisms, *e.g.*, mammals. Examples of

subjects include humans, primates, dogs, cats, mice, rats, and transgenic species thereof. It will be appreciated that specific cell populations may be obtained by isolation from certain tissues by methods known to those skilled in the art. The cells are maintained under conditions necessary to support growth, for example an appropriate temperature (*e.g.*, 37°C) and atmosphere (*e.g.*, air plus 5% CO_2).

The cells are then transfected with the constructs of the present invention by introducing the constructs to the cell population, under conditions favorable for transfection. According to one embodiment of the present invention, cells are treated with compounds that facilitate uptake of the constructs by the cells. According to another embodiment of the present invention, cells are treated with compounds that stimulate cell division and facilitate uptake of the constructs. It will be appreciated that compounds that facilitate uptake of constructs by cells and compounds that stimulate cell division are known to those skilled in the art.

The constructs of the present invention express the transferred gene in a dose dependent manner. The specific dose to be administered to a patient will be determined by the efficacy of the particular construct and/or delivery system employed, the gene transferred, and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular construct or effect a particular patient. In determining the effective amount of the construct or transfected cell to be administered, the physician needs to evaluate circulating plasma levels, toxicities, and progression of disease. It will be appreciated that administration can be accomplished via single or divided doses.

There is a wide variety of suitable formulations for pharmaceutical compositions containing the constructs of the present invention. Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the construct dissolved in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the construct, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. The construct, alone or in combination with other suitable components, may also be made into aerosol formulations to be administered via inhalation, *e.g.*, to the bronchial passageways. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the construct with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the construct with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules or vials. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transfected by the constructs as described above in the context of *ex vivo* therapy can also be administered as described above.

This invention also provides compositions and kits comprising the constructs of the present invention. For example, the composition can comprise the constructs of the present invention in a pharmaceutically acceptable carrier as described above. Kits comprising such compositions and instructions for use are also within the scope of this invention.

In order to more fully demonstrate the advantages arising from the present invention, the following examples are set forth. It is to be understood that the following is by way of example only and is not intended as a limitation on the scope of the invention.

SPECIFIC EXAMPLE 1

I. Methods

Recombinant retroviruses were produced by transient transfection of 293T cells: 2 x 10^6 cells were plated 24 hours before transfection in 60 mm dishes. Transfection was performed by calcium-phosphate precipitation using 3 μg of a retroviral vector (Kinsella, T.M. et al., *Hum. Gene Ther.* 7:1405 (1996)) encoding lucerifase linked to an internal ribosome entry site and a green fluorescent protein derivative (GFP; pEGFP, Clontech), pLZRs-Luc-Gfp, 5 μg of an expression vector

encoding gag and pol, pNGVL-MLVgag-pol, and 1 μg of the envelope encoding plasmid: pNGVL-4070A (ampho) env, pCMV-Eco env or p1012-Ebola GP, respectively. Supernatants corresponding to 24-48 hours post-transfection were harvested, cleared by low-speed centrifugation and either used immediately for infection or frozen at -80°C. Infections were performed in 6-well plates (1.5-2.5 x 10^5 adherent cells) or 12-well plates (5 x 10^5 non-adherent) using different dilutions of the supernatants by incubating the cells overnight with 1 ml and 300 μl, respectively of the diluted supernatants. Polybrene was used at a concentration of 5 μg/ml for all the cell lines except for D17 in which the concentration was 100 μg/ml. After overnight infection, fresh medium was added and the cells were incubated for an additional 24 hours. After infection, the cells were lysed in 25 mM Tris-phosphate pH 8, 2 mM DTT, 2 mM 1,2-diaminocyclohene-N,N,N',N'-tetraacetic acid, 10% glycerol, 1% TritonX-100, and assayed for luciferase activity using Luciferase Assay Reagent (Promega, Madison, WI) in a 1251 BioOrbit Luminometer. The same number of cells (range 5-10 x 10^4) was analyzed for every specific cell line.

Binding of sGP to neutrophils and inverse correlation of binding with activation: *Figures 1A-1A2.* PBMC from normal volunteers were incubated with control or sGP supernatants derived from transfected 293 cells, and immunostaining was performed using a rabbit antibody to sGP as previously described. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996); Xu, L. et al., *Nat. Med.* (1997) in press. Secondary staining was performed with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibody (Sigma, F9887). All incubations were performed at 4°C for 30 minutes with .4 μg of the relevant antibodies per 10^6 cells in a 50 μl volume.

Figures 1B-1B1. Double immunostaining with antibodies to sGP and the neutrophil-specific marker, CD15. Cells were incubated with a FITC conjugated mouse anti-human CD15 antibody (Caltag, cat# MHCD1501), followed by secondary staining with a PE-conjugated anti-rabbit IgG antibody (Sigma) to detect sGP binding. Cells were washed with PBS, fixed in 1% formaldehyde, and analyzed by FACS.

Figure 1C. Specific absorption of sGP by neutrophils. Control or sGP supernatants derived from relevant transfected 293 cells (Xu, L. et al., *Nat. Med.* (1997) in press) were incubated at 1:500 dilution with 10^6 mononuclear or granulocytic cells. Cells were removed and the resulting supernatants analyzed by an 8% SDS PAGE gel. Western blot analysis was performed as previously described (Xu, L. et al., *Nat. Med.* (1997) in press) using an anti-GP rabbit antisera and a secondary

antibody, horseradish peroxidase conjugated donkey anti-rabbit IgG at a dilution of 1:5,000 (Amersham, NA934). Primary antibody was incubated for 30 minutes at room temperature, as was the secondary antibody. The immunocomplexes were detected by chemiluminescence using Supersignal® chemiluminescent substrate reagents (Pierce) according to the manufacturer's instructions. Arrow indicates sGP reactive band.

Infection of different cell types by GP-pseudotyped retroviral vector and preferential binding to endothelial cells: *Figure 2A.* Infection of different indicator cell lines with the Ebola-GP pseudotyped retrovirus expressing luciferase. Amphotropic and ecotropic retroviral vectors were used as controls. Viruses were diluted to different multiplicities of infection (MOI) to provide for equal luciferase activity on Hela cervical epithelial cells, permissive for amphotropic retrovirus, D17 dog osteosarcoma cells (Embretson, J.E. et al., *J. Virol.* 61:3454 (1987)), which are permissive for amphotropic, xenotropic, and ecotropic retroviruses, and BW5147 T leukemia cells permissive for amphotropic and ecotropic virus. In these groups, GP virus titer was 1-4x10^5/ml and amphotropic virus was ~2x10^4/ml (MOI's $\approx$ 1.0 and 0.1, respectively), and the ecotropic virus titer was ~10^6/ml (MOI $\approx$ 10). Titers were determined by endpoint dilution of reporter activity of the amphotropic virus in D17 cells, and was normalized to reverse transcriptase activity for the GP virus.

Figure 2B. Analysis of different normal or transformed cell lines by infection with amphotropic or GP retroviral vectors at the same titer (10^4/ml, MOI $\approx$ 0.2). Forty-eight hours after infection, an equivalent of 5 x 10^4 cells was assayed for luciferase activity after exposure to equal titers of viral stocks. Luminescence is expressed as the fold-increase over non-infected control cells.

Figures 2C-2C3. The binding of sGP (left) or GP-pseudotyped retrovirus (right) to neutrophils (upper panel) or microvascular endothelium (lower panel) was determined by FACS. sGP binding was performed as in Fig. 1A, and retrovirus incubation was performed at 37°C for 2 hours in the presence of polybrene (8 μg/ml).

Figure 2D. Infection of D17 cells by GP-pseudotyped virus in the absence (lane 1, none) or presence of control (lane 2) or sGP supernatant (lane 3) from transfected 293 cells. Gene transfer was measured by the luciferase assay as described below. Luminescence refers to relative light units in the luciferase assay.

Depend nce of sGP binding n CD16b and correlation of binding with neutrophil activation: *Figures 3A-3D.* Neutrophils were incubated for 30 minutes at 4°C with a mouse antibody to CD16b (upper panel; clone 3G8 from Immunotech,

cat# 1M0813) or CD62L (middle panel, R&D Systems), compared to the indicated control antibody [purified mouse IgG (Vector Laboratories), I-2000], followed by supernatants from control or sGP-transfected 293 cells, primary rabbit antibody to sGP, and a FITC-conjugated secondary antibody to rabbit IgG (Fig. 1, legend). Cells were washed with PBS, fixed in 1% formaldehyde, and analyzed by FACS. For blocking, 10^6 cells were incubated with 0.5 – 1 μg of the relevant antibodies for 30 minutes in a 50 μl volume.

Figures 3E-3F. Immunostaining with sGP was performed on isolated neutrophils which were maintained in media (none) or incubated with PMA (10 ng/ml) at 37°C for 30 minutes (PMA).

Effect of sGP on neutrophil function: *Figures 4A-4B.* Exposure of neutrophils to sGP inhibits down modulation of L-selectin. Isolated neutrophils were incubated with the indicated control or sGP containing supernatants (Xu, L. et al., *Nat. Med.* (1997) in press) and defined media (AIM V, GIBCO) for 4 hours at 37°C. Expression of L-selectin was determined using an anti-CD62L antibody (R&D Systems), followed by the secondary staining using a FITC-conjugated anti-mouse IgG (Sigma, F2883) as described in Fig. 1, legend. Cells were washed with PBS, fixed with 1% formaldehyde and analyzed by FACS for relative levels of fluorescence intensity as a function of cell number. An isotype control was used to quantitate background levels of immunostaining (neg.). Results are representative of three independent experiments.

II. Results

To determine the specificity of Ebola virus glycoproteins, expression vectors encoding either sGP, GP, or a plasmid control (Xu, L. et al., *Nat. Med.* (1997) in press) were transfected into 293 cells, and cell culture supernatants were used as a source of relevant recombinant glycoproteins. Binding of sGP was determined by immunofluorescence analysis after incubation of relevant supernatants with normal or transformed human cell lines. No binding was detected to several hematopoietic lineages, including lymphocytes or monocytes (Fig. 1A), or transformed Jurkat or CEM T leukemias, the HL60 myelomonocytic or U937 promonocytic leukemia cells. In contrast, sGP was able to bind to granulocytic cells, as evidenced by FACS analysis of this subset of peripheral blood mononuclear cells (PBMC) discriminated by cell size and granularity (Fig. 1A). This cell specificity was confirmed by using double-staining with a granulocyte-specific cell surface marker, CD15 (Fig. 1B). Absorption of sGP

by purified neutrophils in the absence of antibodies also resulted in depletion of sGP, indicating that binding to the neutrophil occurred in the absence of antibody (Fig. 1C).

A potential structural similarity between Ebola GP and avian sarcoma virus envelope protein has been previously proposed (Gallaher, W.R., *Cell* 85:477 (1996)), raising the possibility that this protein could be incorporated into retroviral particles. To determine the binding specificity of the transmembrane glycoprotein, pseudotyping of a Moloney leukemia virus was therefore attempted. Infectivity of different cell types by this pseudotyped vector was determined with a luciferase reporter gene. de Wet, J.R. et al., *Mol. Cell. Biol.* 7:725 (1987). This analysis revealed infection of cells different from those which interacted with sGP (Fig. 2A,B). For example, though it could infect other cell types, transduction by the GP retroviral vector readily occurred in endothelial cells, either from the microvasculature (MVEC) or umbilical veins (HUVEC) (Fig. 2B), which did not bind sGP (Fig. 2C, left). When the specificity of GP-retrovirus was compared to murine retroviruses pseudotyped with amphotropic or ecotropic envelope gp70 proteins, the range of susceptible target cells differed (Fig. 2B), suggesting that the virus receptor(s) for Ebola GP differ from those previously described for gp70. Minimal binding of GP-virus was observed on neutrophils, despite the ability of these cells to bind sGP (Fig. 2C, upper panel) and the fact that immunoreactive protein was detected on the virus. Conversely, GP-virus binding to endothelial cells was readily detected, though these cells did not bind sGP (Fig. 2C, lower panel). When sGP was analyzed for its effect on GP retroviral gene transfer, infection was not inhibited by sGP (Fig. 2D), further confirming the divergent specificities of the two forms of the viral glycoprotein. Recent studies have revealed that the biochemical forms of these proteins differ, with sGP present in solution primarily as a homodimer and GP as a trimer, suggesting that differences in multimer composition may contribute to these alternative specificities.

Potential cell surface receptors for sGP were analyzed with antibodies to several neutrophil cell surface antigens to interfere with sGP binding, including CD15, L-selectin (CD62L), CD16b, and several common leukocyte antigens. Only the neutrophil-specific form of the low affinity F_c γreceptor III, CD16b, inhibited sGP binding specifically. Antibodies to CD62L, for example, did not inhibit sGP binding (Fig. 3). Binding to neutrophils correlated with their activation state and CD16b expression since no binding was observed in cells stimulated with phorbol 12-myristate 13-acetate (PMA) for 30 minutes, at which time CD16b expression was markedly decreased on these cells (Fig. 3, lower panel). Overexpression of this form

of CD16 on a heterologous cell type, 3T3 fibroblasts, did not confer sGP binding to these cells by FACS analysis, suggesting that CD16b is necessary but not sufficient for stable binding.

Binding of sGP did not inhibit neutrophil activation in response to potent pleiotropic activators (PMA, IL-8, or f-Met-Leu-Phe), as measured by down modulation of L-selectin expression using FACS analysis. In a defined serum-free medium, partial activation of neutrophils was observed, with a decrease in L-selectin expression at 4 hours (Fig. 4). Under these conditions, incubation of neutrophils with sGP supernatant prevented this decrease in L-selectin expression (Fig. 4). Because L-selectin was not required for sGP binding (Fig. 3), this effect was apparently indirect, through a mechanism not yet defined, possibly involving CD16b or carbohydrate interactions of the highly glycosylated sGP protein.

The expression of alternative Ebola virus glycoproteins in clinical infection has long been recognized, but their functional roles and cell specificity have not been defined. Early after infection, high levels of the secreted protein are found in the serum and precede fulminant replication and dissemination of virus systemically, at which time synthesis of transmembrane GP is markedly increased. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996). The inventors have now found that the binding specificities of these two molecules differ. It had been proposed that sGP may serve as a decoy to prevent recognition of GP, possibly to temporarily inhibit virus binding to target cells. The studies set forth herein suggest that this hypothesis is unlikely to be correct. The binding specificities of these proteins differ, and despite the fact that they are derived from the same viral gene, it has been surprisingly found that alternative forms of the glycoprotein have been selected for different functions.

Although these proteins share identical amino terminal sequences, their carboxyl terminal regions differ. Sanchez, A. et al., *Virus Res.* 29:215 (1993). These sequences are likely responsible for the differences in binding specificity, either through direct interactions in these domains or by their effect on multimerization. The secreted glycoprotein binds to neutrophils to prevent early events in activation, possibly serving to diminish any inflammatory responses which might provide innate immunity to the virus, facilitating productive viral replication. The subsequent increase in GP synthesis gives rise to virus which in turn could infect other cells. Filoviruses have been shown previously to infect and replicate in different cell types and appear to grow readily in endothelial cells *in vivo*. Peters, C.J. et al., *Fields Virology*, B.N. Fields, D.M. Knipe and P.M. Howley, Eds. (Lippincott-Raven, Philadelphia) (1996);

Schnittler, H.J. et al., *J. Clin. Invest.* 91:1301 (1993). The findings set forth herein suggest that its tropism for this cell type is probably determined by the specificity of GP. In Ebola infection, preferential binding and infection of microvascular endothelial cells may lead ultimately to a loss of capillary integrity that results in the severe hemorrhage observed in the terminal stages of this disease. The differential binding of these two gene products from the same viral structural gene generated by RNA editing suggests that they have evolved functionally to differentially affect immunity and infectivity. The ability to facilitate viral replication and target the virus to endothelial cells by alternative products of the same viral gene represents an efficient genetic mechanism which can account for different pathologic features of this disease. Inhibition of sGP binding to neutrophils and GP to endothelium is likely to ameliorate the effects of acute Ebola virus infection.

SPECIFIC EXAMPLE 2

I. Methods

Production of pseudotyped MuLV retroviruses expressing green fluorescent protein (GFP): 50% - 70% confluent 293 T cells in 60mm tissue culture dishes were transfected using the calcium phosphate method and the following plasmids: 0.3 μg 1012 GP(Z) (see Figure 8) or 1012 sGp-Gp(Z) (see Figure 9), 3 μg LZR-gfp, 2 μg pNGVL-gag-pol. After overnight transfection, fresh media was added to cells. Twenty hours later, the supernatants were harvested and filtered through a .45 μm filter.

Infection of HUVEC cells using the pseudotyped retroviruses: The day before infection, 30% - 50% confluent HUVEC cells were prepared in 6-well plates. 1 ml of pseudotyped retroviral supernatant was added to one well of the 6-well plates with 15 μg/ml of polybrene. Sixteen hours later, the viruses were removed and normal media was added. After 24 hours, the cells were lifted and GFP expression measured using FACS analysis.

Construction of 1012 sGP-GP(Z): 1012 sGP(Z) cells were digested with PstI and treated with Klenow, then digested with XbaI. 1012 GP(Z) cells were digested by EcoRI and treated with Klenow, then digested with KpnI. PstI/Klenow/XbaI treated sGP fragment and EcoRI/Klenow/KpnI treated GP fragment were then cloned into XbaI/KpnI treated pVR-1012 plasmid.

GP and sGP derivatives: The receptor recognition domain, mucin-like domain and/or TM domain of GP and sGP were mutated. The mutated GP and sGP was then tested for its ability to pseudotype and for cytotoxicity in producer cells.

II. Results

To determine the efficacy of targeting endothelium with the gene transfer vectors pseudotyped with GP of the present invention, HUVEC cells were infected with GP(Z) pseudotyped MuLV retrovirus (LZR-gfp) and sGP-GP(Z) pseudotyped MuLV retrovirus (LZR-gfp). Figures 5A-5C show the infection rate (GFP expression) measured using FACS analysis. As shown in Figure 5B, the GP(Z) pseudotyped MuLV retrovirus (LZR-gfp) was effective in targeting and expressing GFP in endothelium.

To determine whether mutating GP would effect its ability to pseudotype and/or decrease toxicity in producer cells, the receptor recognition domain, mucin-like domain and/or TM domain were mutated. Figure 10 shows the results. The optimal envelope is able to pseudotype but shows minimal toxicity.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

All patents and other references cited herein are incorporated by reference as if fully set forth.

WE CLAIM:

1. A genetic construct comprising a gene operatively-linked to a carrier, wherein the carrier is associated with a transmembrane form of viral glycoprotein or derivative thereof.

2. The genetic construct of Claim 1, wherein the transmembrane form of viral glycoprotein or derivative thereof is expressed on the surface of the carrier.

3. The genetic construct of Claim 1, wherein the transmembrane form of viral glycoprotein or derivative thereof is from Ebola.

4. The genetic construct of Claim 1, wherein the carrier is a viral vector.

5. The genetic construct of Claim 1, wherein the carrier is a non-biologic gene targeting vehicle.

6. The genetic construct of Claim 4, wherein the viral vector is a retroviral vector.

7. The genetic construct of Claim 4, wherein the viral vector is a lentiviral vector.

8. The genetic construct of Claim 5, wherein the non-biologic gene targeting vehicle is a liposome.

9. The genetic construct of Claim 5, wherein the non-biologic gene targeting vehicle is a DNA-protein complex.

10. A method of targeting a gene to a cell comprising the step of administering to a cell population a genetic construct comprising the gene operatively-linked to a carrier, wherein the carrier is associated with a transmembrane form of viral glycoprotein or derivatives thereof.

11. The method of Claim 10, wherein the transmembrane form of viral glycoprotein or derivative thereof is expressed on the surface of the carrier.

12. The method of Claim 10, wherein the transmembrane form of viral glycoprotein or derivative thereof is from Ebola.

13. The method of Claim 10, wherein the carrier is a viral vector.

14. The method of Claim 10, wherein the step of administration is *ex vivo*.

15. The method of Claim 10, wherein the step of administration is *in vivo*.

16. The method of Claim 10, wherein the cell is an endothelial cell.

17. The method of Claim 10, wherein the cell is a hepatocyte.

18. The method of Claim 10, wherein the cell is a monocyte.

19. The method of Claim 10, wherein the cell is a dendritic cell.

20. The method of Claim 14, further comprising the step of introducing the cell population to a subject.

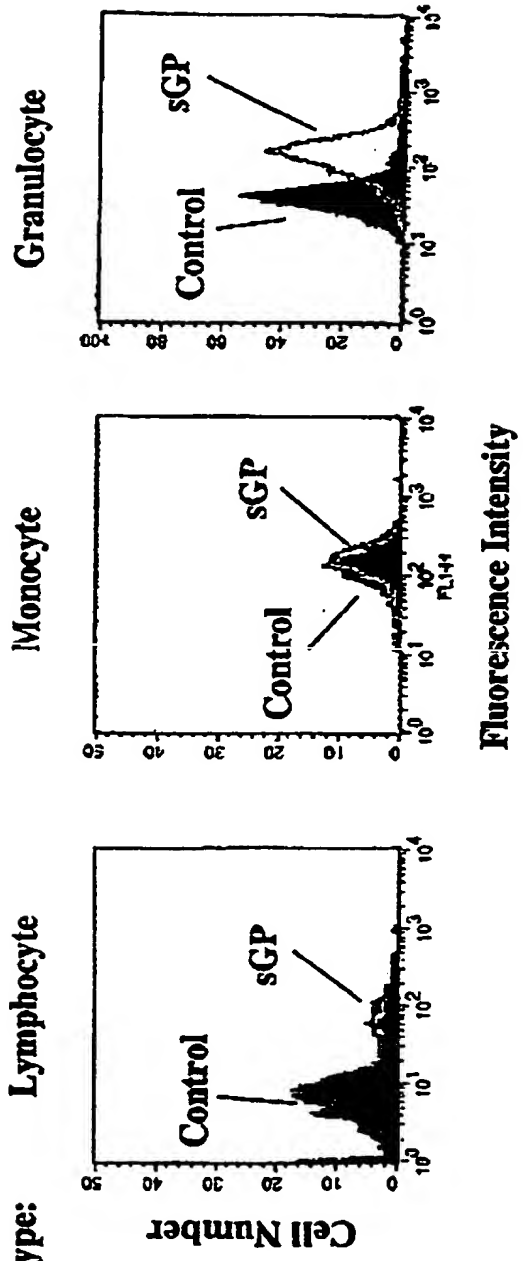


Figure 1A

Figure 1A1

Figure 1A2

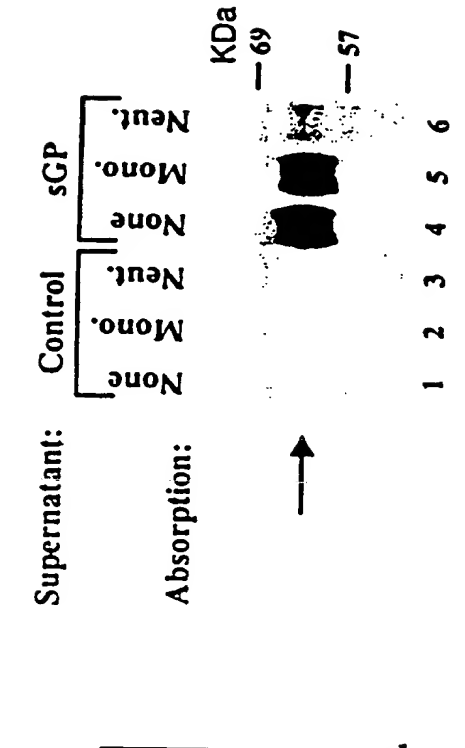


Figure 1C

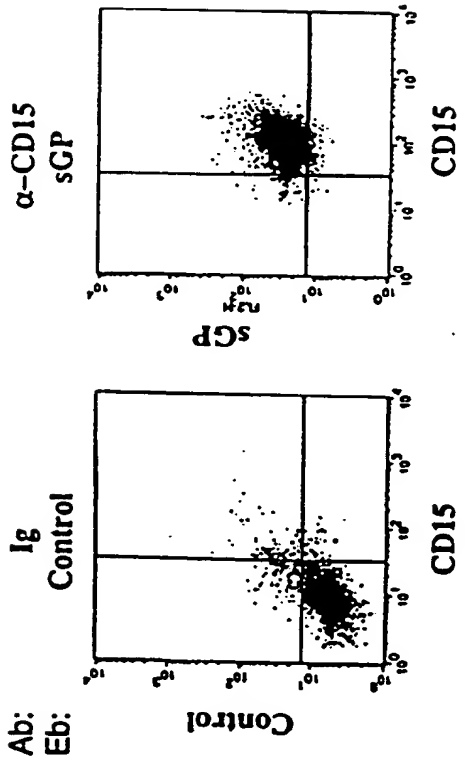


Figure 1B

Figure 1B1

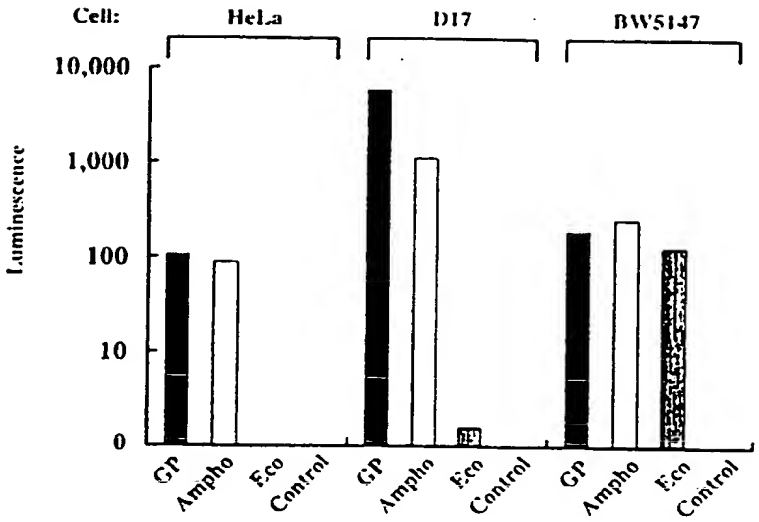


Figure 2A

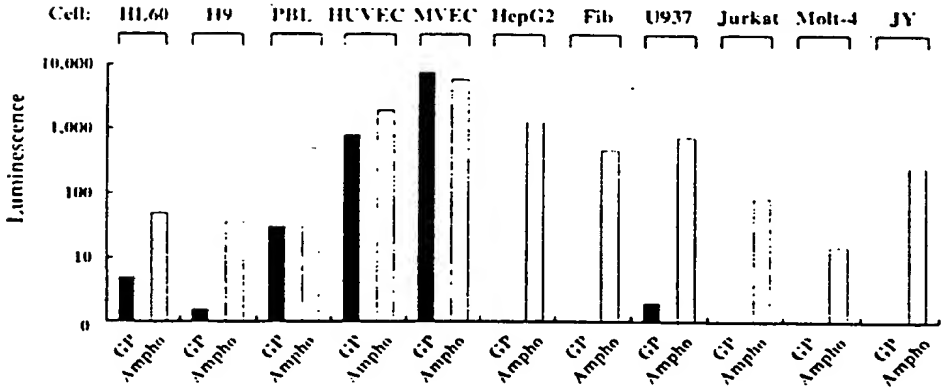


Figure 2B

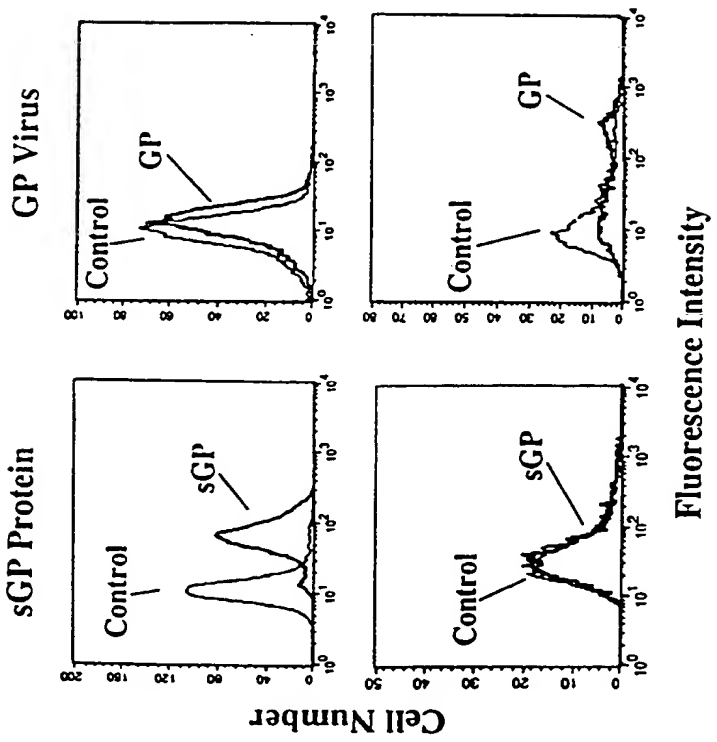


Figure 2C

Figure 2C1

Figure 2C2

Figure 2C3

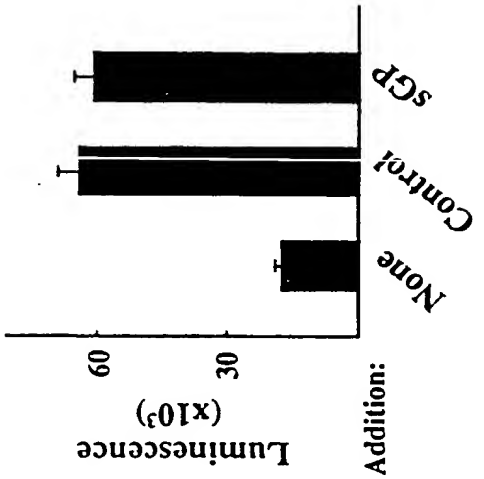


Figure 2D

Figure 3A

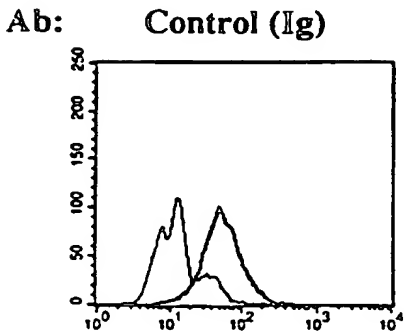


Figure 3B

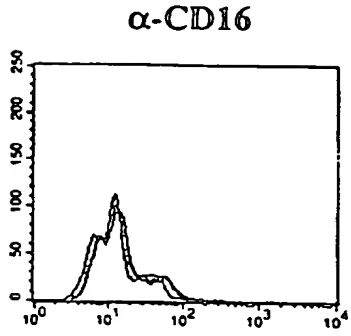


Figure 3C

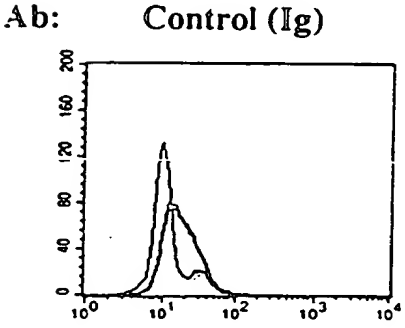
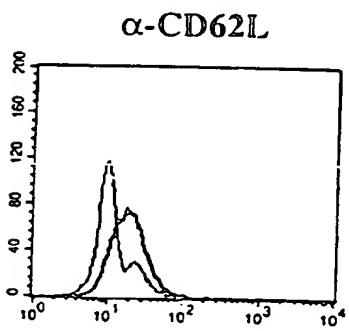


Figure 3D



Cell Number

Figure 3E

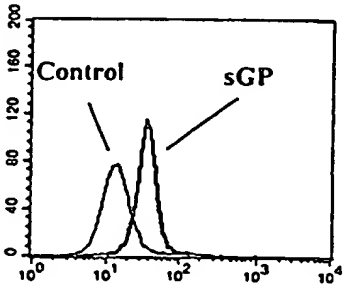
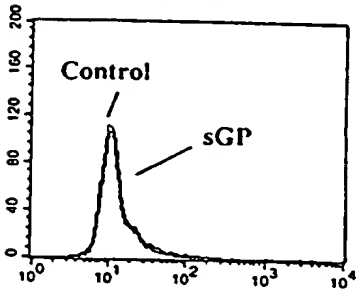


Figure 3F



Fluorescence Intensity

Figure 4A

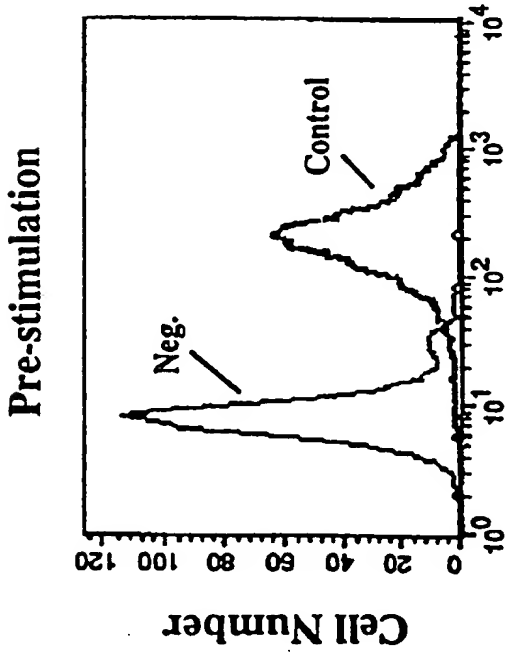


Figure 4B

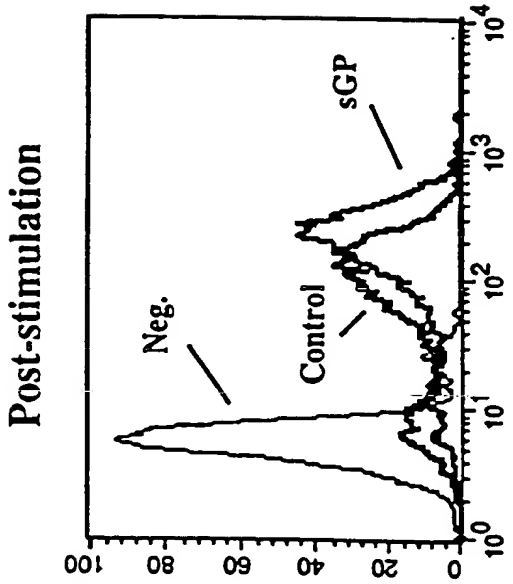


Figure 5A

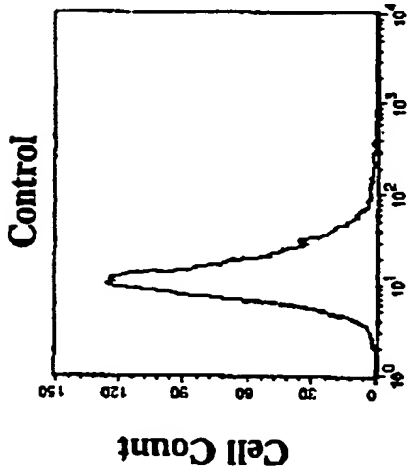


Figure 5B

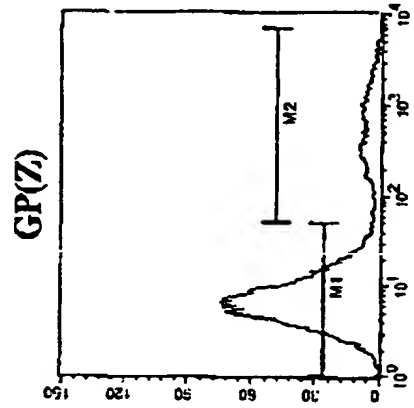
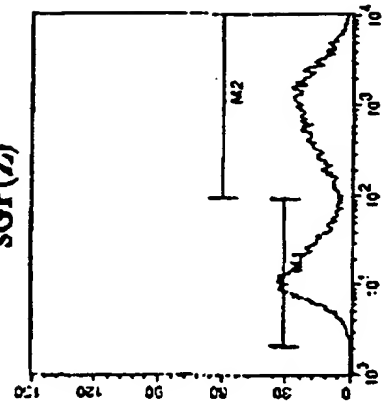
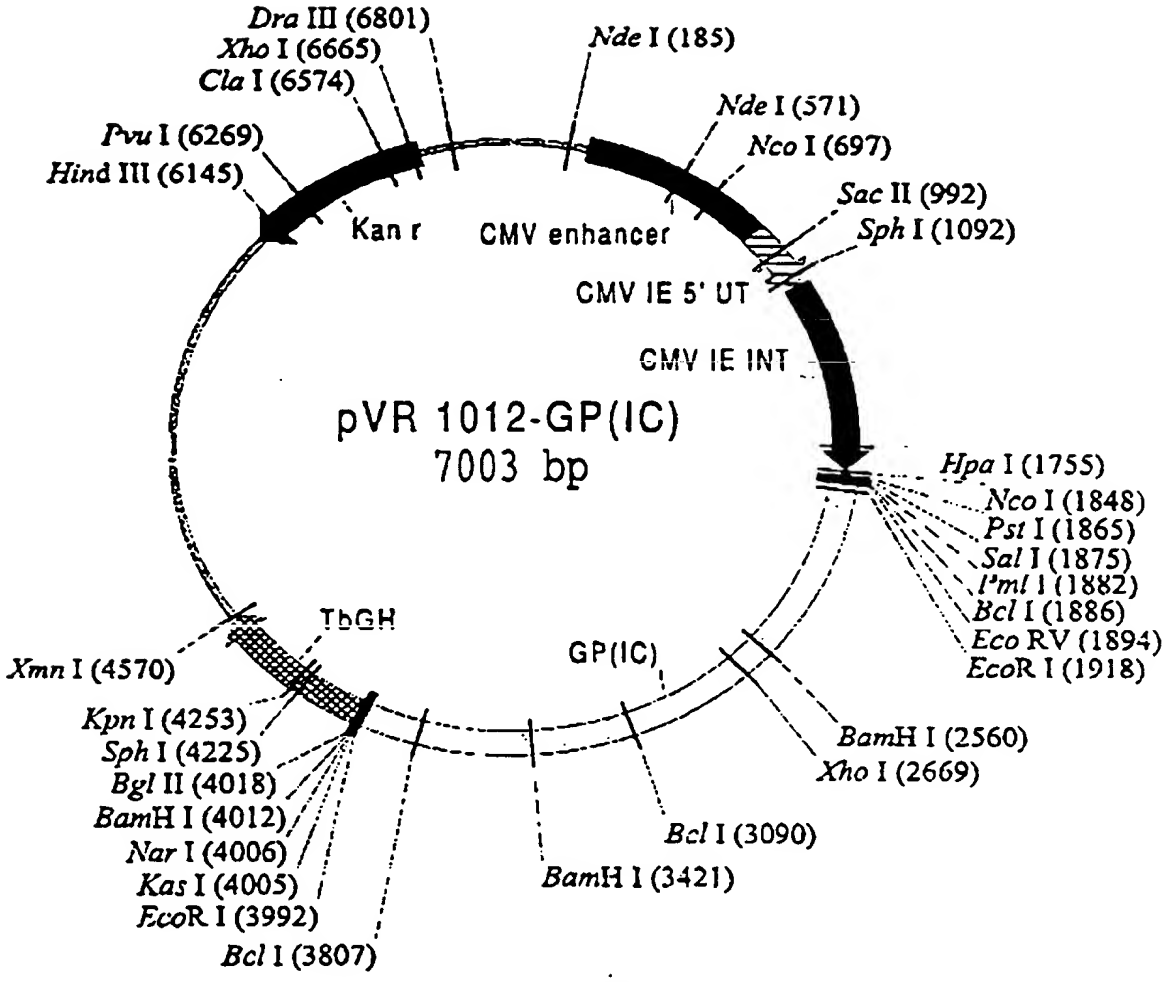


Figure 5C

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Xho I (6665)
Cla I (6574)
Pvu I (6269)
Hind III (6145)
Nde I (185)
Nde I (571)
Nco I (697)
Sac II (992)
Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
pVR 1012-GP(IC)
7003 bp
Hpa I (1755)
Nco I (1848)
Pst I (1865)
Sal I (1875)
I'ml I (1882)
Bcl I (1886)
Eco RV (1894)
EcoR I (1918)
TbGH
GP(IC)
Xmn I (4570)
Kpn I (4253)
Sph I (4225)
Bgl II (4018)
BamH I (4012)
Nar I (4006)
Kas I (4005)
EcoR I (3992)
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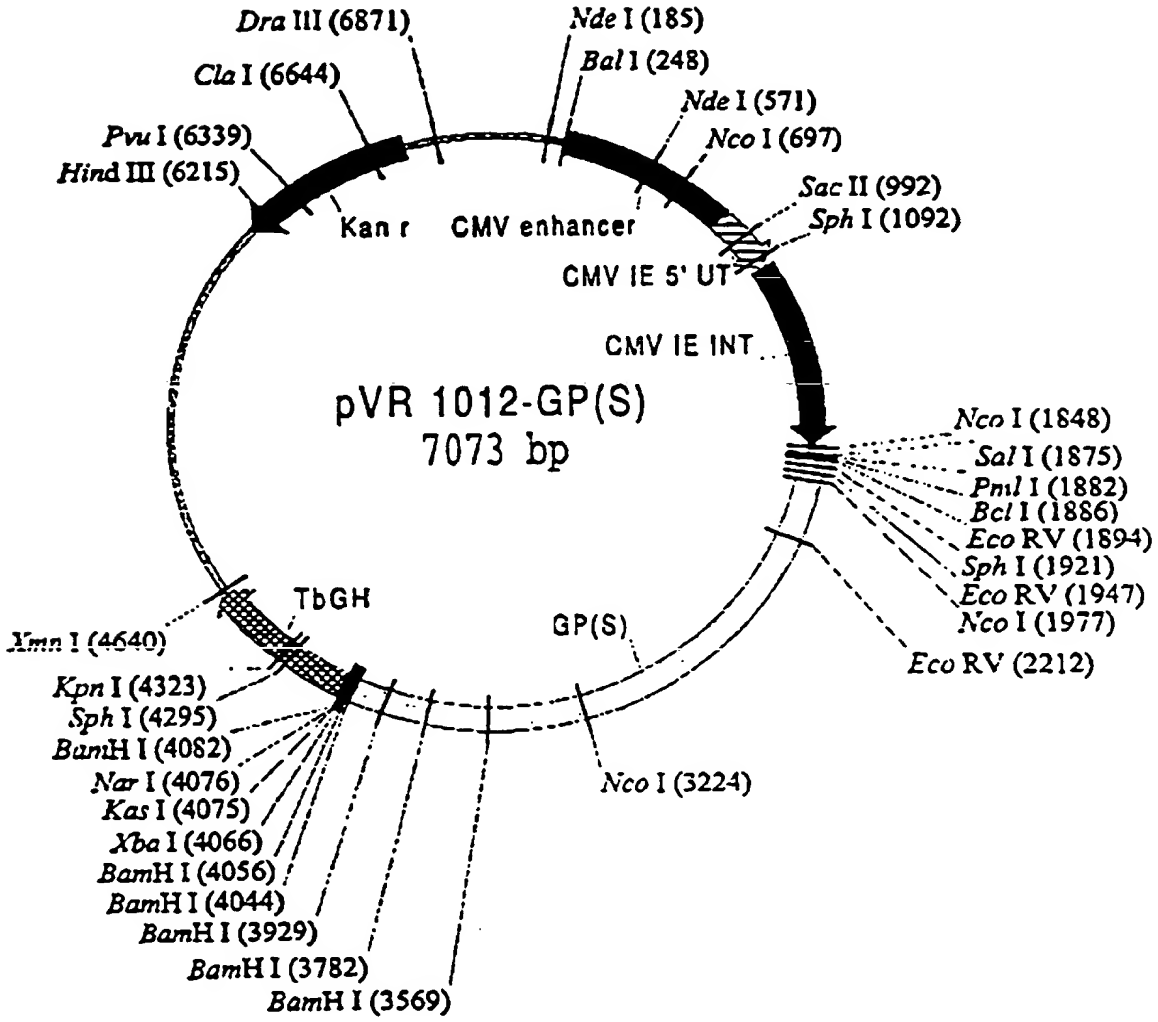
Xho I (2669)
Bcl I (3090)
BamH I (3421)

Figure 6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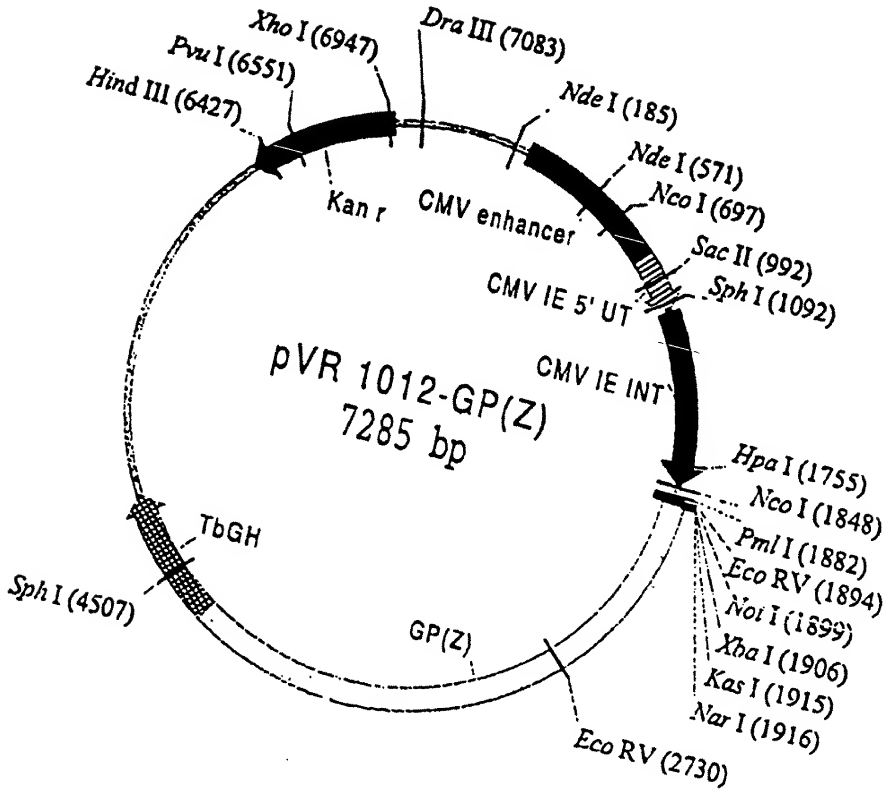
Sac II (992)
Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
pVR 1012-GP(S)
7073 bp
Nco I (1848)
Sal I (1875)
Pml I (1882)
Bcl I (1886)
Eco RV (1894)
Sph I (1921)
Eco RV (1947)
Nco I (1977)
Eco RV (2212)
TbGH
GP(S)
Xmn I (4640)
Kpn I (4323)
Sph I (4295)
BamH I (4082)
Nar I (4076)
Kas I (4075)
Xba I (4066)
BamH I (4056)
BamH I (4044)
BamH I (3929)
BamH I (3782)
BamH I (3569)
Nco I (3224)

Figure 7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Kan r
CMV enhancer
Sac II (992)
Sph I (1092)
CMV IE 5' UT
pVR 1012-GP(Z)
7285 bp
CMV IE INT
Hpa I (1755)
Nco I (1848)
Pml I (1882)
Eco RV (1894)
Not I (1899)
Xba I (1906)
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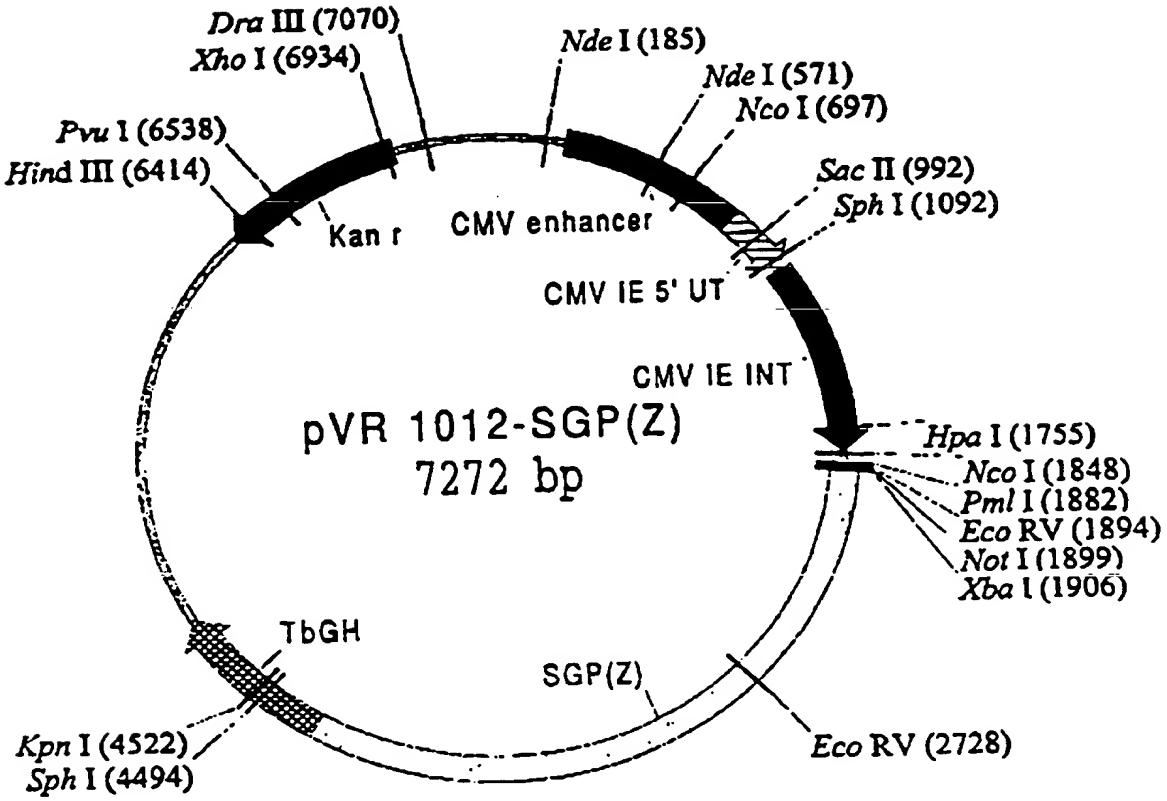
Nar I (1916)
TbGH
Sph I (4507)
GP(Z)
Eco RV (2730)

Figure 8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Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
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7272 bp
Hpa I (1755)
Nco I (1848)
Pml I (1882)
Eco RV (1894)
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TbGH
SGP(Z)
Kpn I (4522)
Sph I (4494)
Eco RV (2728)

Figure 9

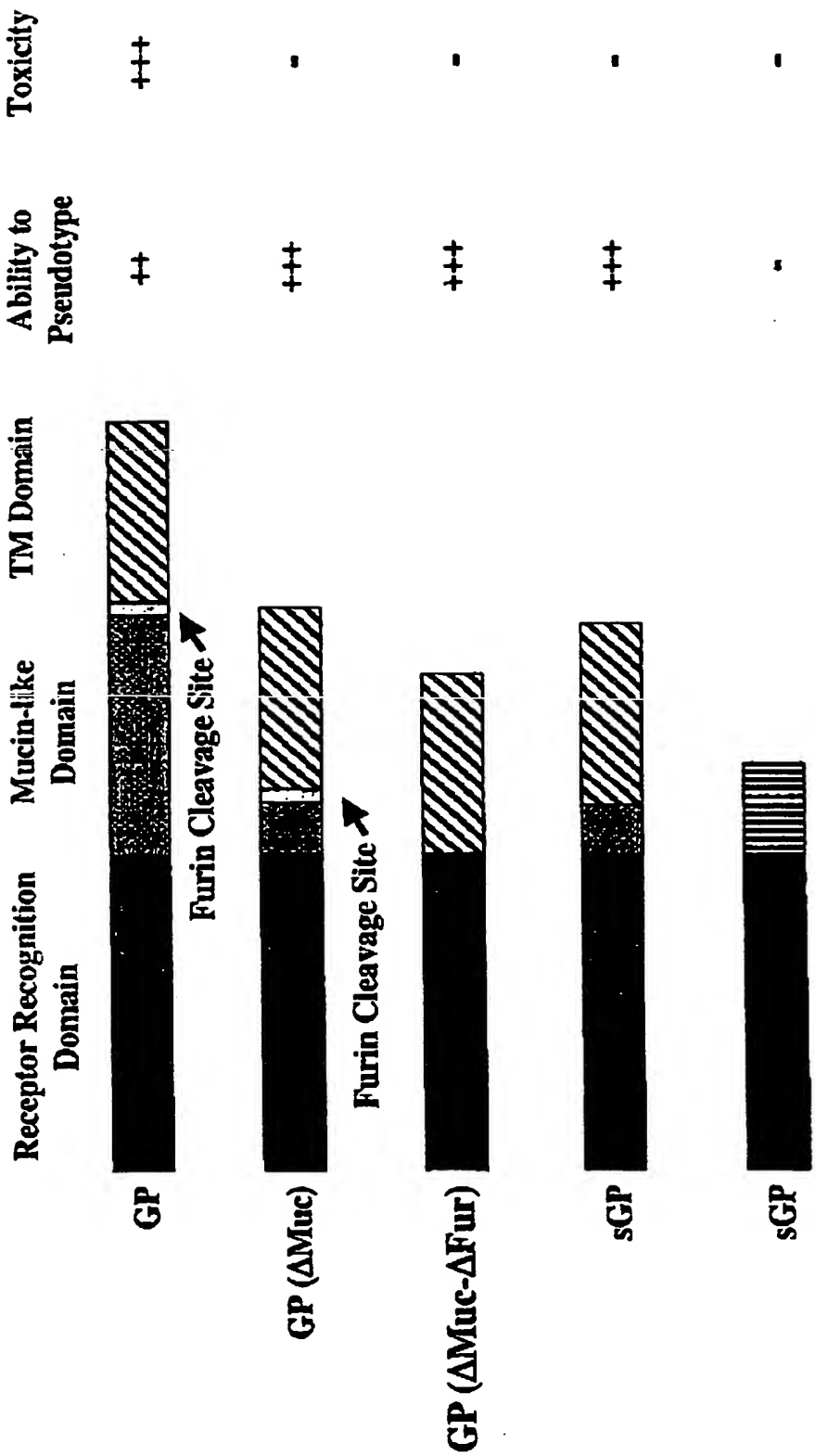
Receptor Recognition Domain
Mucin-like Domain
TM Domain
Furin Cleavage Site
Furin Cleavage Site
GP
GP (ΔMuc)
GP (ΔMuc-ΔFur)
sGP
sGP
Ability to Pseudotype
++
+++
+++
+++
-
Toxicity
+++
-
-
-
-

Figure 10

SEQUENCE LISTING ID NO: 1

pVR 1012-GP(IC)

General Description

DNA pVR 1012-GP(IC)
Local object
Created: 09/14/98 04:17PM
Last Modification Date: ? (no data)
length: 7003 bp
storage type: Basic
form: Circular

Comments

Restriction Map

BglII: 1 site AGATCT / TCTAGA

ClaI: 1 site ATCGAT / TAGCTA

DraIII: 1 site CACNNNGTG / GTGNNNCAC

EcoRV: 1 site GATATC / CTATAG

HindIII: 1 site AAGCTT / TTCGAA

HpaI: 1 site GTTAAC / CAATTG

KasI: 1 site GGCGCC / CCGCGG

KpnI: 1 site GGTACC / CCATGG

NarI: 1 site GGCGCC / CCGCGG

PmlI: 1 site CACGTG / GTGCAC

PstI: 1 site CTGCAG / GACGTC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

SalI: 1 site GTCGAC / CAGCTG

XmnI: 1 site GAANNNNTTC / CTTNNNNAAG

EcoRI: 2 sites GAATTC / CTTAAG

NcoI: 2 sites CCATGG / GGTACC

NdeI: 2 sites CATATG / GTATAC

SphI: 2 sites GCATGC / CGTACG

XhoI: 2 sites CTCGAG / GAGCTC

BamHI: 3 sites GGATCC / CCTAGG

BclI: 3 sites TGATCA / ACTAGT

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1840

TbGH
Start: 4020 End: 4572

Kan r
Start: 6068 End: 6690 (Complementary)

Misc_feature (2 signals)

CMV enhancer
Start: 248 End: 885

GP(IC)
Start: 1870 End: 4019

Annotations

```
   1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
      AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

  51  GAGACCGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
      CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

 101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
      AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                        NdeI
 151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
      GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

 201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
      GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

 251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
      AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

 301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
      AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

 351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
      TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

 401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
      TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

 451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
      GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

 501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
      GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                          NdeI
 551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
      CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

 601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
      ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                      NcoI
 651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
      TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

      NcoI
 701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
      CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

 751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
      TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

 801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
      CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
```

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 851  TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 ....................................................................................

 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
 ....................................................................................

                                                 SacII
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 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
 ....................................................................................

1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
 ....................................................................................

                                                 SphI
                                                 ====

1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
 ....................................................................................

1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
 ....................................................................................

1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
 ....................................................................................

1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
 ....................................................................................

1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
 ....................................................................................

1301  ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
 ....................................................................................

1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
 ....................................................................................

1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
 ....................................................................................

1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
 ....................................................................................

1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
 ....................................................................................

1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
 ....................................................................................

1601  TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
 ....................................................................................

1651  CCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      GGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
 ....................................................................................

1701  CCGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
 ....................................................................................
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HpaI

1751 TGTTAACGGT GGAGCGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
ACAATTGCCA CCTCGCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

NcoI

1801 CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

SalI

NcoI PstI PmlI BclI EcoRV

1851 GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

EcoRI

1901 CCGCGCGGCC GCTCTAGAAT TCTCTAATCA CAGTCATCAT GGGAGCGTCA
GGCGCGCCGG CGAGATCTTA AGAGATTAGT GTCAGTAGTA CCCTCGCAGT

1951 GGGATTCTGC AATTGCCCCG TGAGCGCTTC AGGAAAACAT CTTTCTTTGT
CCCTAAGACG TTAACGGGGC ACTCGCGAAG TCCTTTTGTA GAAAGAAACA

2001 TTGGGTAATA ATCCTATTCC ATAAAGTCTT TTCAATCCCG TTGGGGGTTG
AACCCATTAT TAGGATAAGG TATTTCAGAA AAGTTAGGGC AACCCCCAAC

2051 TACACAACAA TACCCTACAA GTGAGTGATA TTGACAAGTT TGTGTGCCGA
ATGTGTTGTT ATGGGATGTT CACTCACTAT AACTGTTCAA ACACACGGCT

2101 GACAAACTCT CTTCAACTAG CCAATTGAAG TCAGTCGGGT TGAACTTGGA
CTGTTTGAGA GAAGTTGATC GGTTAACTTC AGTCAGCCCA ACTTGAACCT

2151 GGGCAATGGA GTAGCAACTG ATGTACCAAC GGCAACCAAA AGATGGGGTT
CCCGTTACCT CATCGTTGAC TACATGGTTG CCGTTGGTTT TCTACCCCAA

2201 TTCGAGCTGG TGTTCCACCA AAGGTGGTAA ATTACGAAGC TGGAGAATGG
AAGCTCGACC ACAAGGTGGT TTCCACCATT TAATGCTTCG ACCTCTTACC

2251 GCTGAGAACT GTTATAACCT GGCTATAAAG AAAGTTGATG GTAGTGAGTG
CGACTCTTGA CAATATTGGA CCGATATTTC TTTCAACTAC CATCACTCAC

2301 CCTACCAGAA GCCCCTGAGG GAGTGAGGGA TTTTCCCCGT TGCCGCTATG
GGATGGTCTT CGGGGACTCC CTCACTCCCT AAAAGGGGCA ACGGCGATAC

2351 TACACAAAGT CTCAGGAACT GGACCATGCC CAGGAGGACT CGCCTTTCAC
ATGTGTTTCA GAGTCCTTGA CCTGGTACGG GTCCTCCTGA GCGGAAAGTG

2401 AAAGAAGGAG CCTTCTTCCT GTATGACCGA CTCGCATCAA CAATCATTTA
TTTCTTCCTC GGAAGAAGGA CATACTGGCT GAGCGTAGTT GTTAGTAAAT

2451 TCGGGGTACA ACCTTTGCCG AAGGAGTTAT TGCATTTCTG ATCTTGCCTA
AGCCCCATGT TGGAAACGGC TTCCTCAATA ACGTAAAGAC TAGAACGGAT

2501 AGGCGCGAAA GGATTTTTTC CAGTCTCCTC CATTGCATGA GCCTGCCAAC
TCCGCGCTTT CCTAAAAAAG GTCAGAGGAG GTAACGTACT CGGACGGTTG

BamHI

2551 ATGACCACGG ATCCCTCCAG TTACTATCAC ACGACAACAA TAAACTACGT
TACTGGTGCC TAGGGAGGTC AATGATAGTG TGCTGTTGTT ATTTGATGCA

2601 GGTTGATAAT TTTGGAACCA ACACCACAGA GTTTCTGTTC CAAGTCGATC
CCAACTATTA AAACCTTGGT TGTGGTGTCT CAAAGACAAG GTTCAGCTAG

XhoI

2651 ATTTGACGTA TGTGCAGCTC GAGGCAAGAT TCACACCACA ATTCCTTGTC
TAAACTGCAT ACACGTCGAG CTCCGTTCTA AGTGTGGTGT TAAGGAACAG

2701 CTCCTAAATG AAACCATCTA CCCTGATAAC CGCAGAAGTA ACACAACAGG
GAGGATTTAC TTTGGTAGAT GAGACTATTG GCGTCTTCAT TGTGTTGTCC

2751 AAAACTAATC TGGAAAATAA ATCCCACTGT TGATACCAGC ATGGGTGAGT
TTTTGATTAG ACCTTTTATT TAGGGTGACA ACTATGGTCG TACCCACTCA

2801 CGGCTTTCTG GGAAAATAAA AAAACTCCAC AAAAACCCTT TCAAGTGAAG
CCCGAAAGAC CCTTTTATTT TTTTGAAGTG TTTTTGGGAA AGTTCACTTC

2851 AGTTGTCTTT CGTACCTGTA CCAGAAACCC AGAACCAGGT CCTTGACACG
TCAACAGAAA GCATGGACAT GGTCTTTGGG TCTTGGTCCA GGAACTGTGC

2901 ACAGCGACGG TCTCTCCTCC CATCTCCGCC CACAACCACG CAGGCGAAGA
TGTCGCTGCC AGAGAGGAGG GTAGAGGCGG GTGTTGGTGC GTCCGCTTCT

2951 CCACAAAGAA TTGGTTTCAG AGGATTCCAC TCCAGTGGTT CAGATGCAAA
GGTGTTTCTT AACCAAAGTC TCCTAAGGTG AGGTCACCAA GTCTACGTTT

3001 ACATCAAGGG AAAGGACACA ATGCCAACCA CAGTGACGGG TGTACCAACA
TGTAGTTCCC TTTCCTGTGT TACGGTTGGT GTCACTGCCC ACATGGTTGT

BclI

3051 ACCACACCCT CTCCATTTCC AATCAATGCT CGCAACACTG ATCATACCAA
TGGTGTGGGA GAGGTAAAGG TTAGTTACGA GCGTTGTGAC TAGTATGGTT

3101 ATCATTTATC GGCCTGGAGG GGCCCCAAGA AGACCACAGC ACCACACAGC
TAGTAAATAG CCGGACCTCC CCGGGGTTCT TCTGGTGTCG TGGTGTGTCG

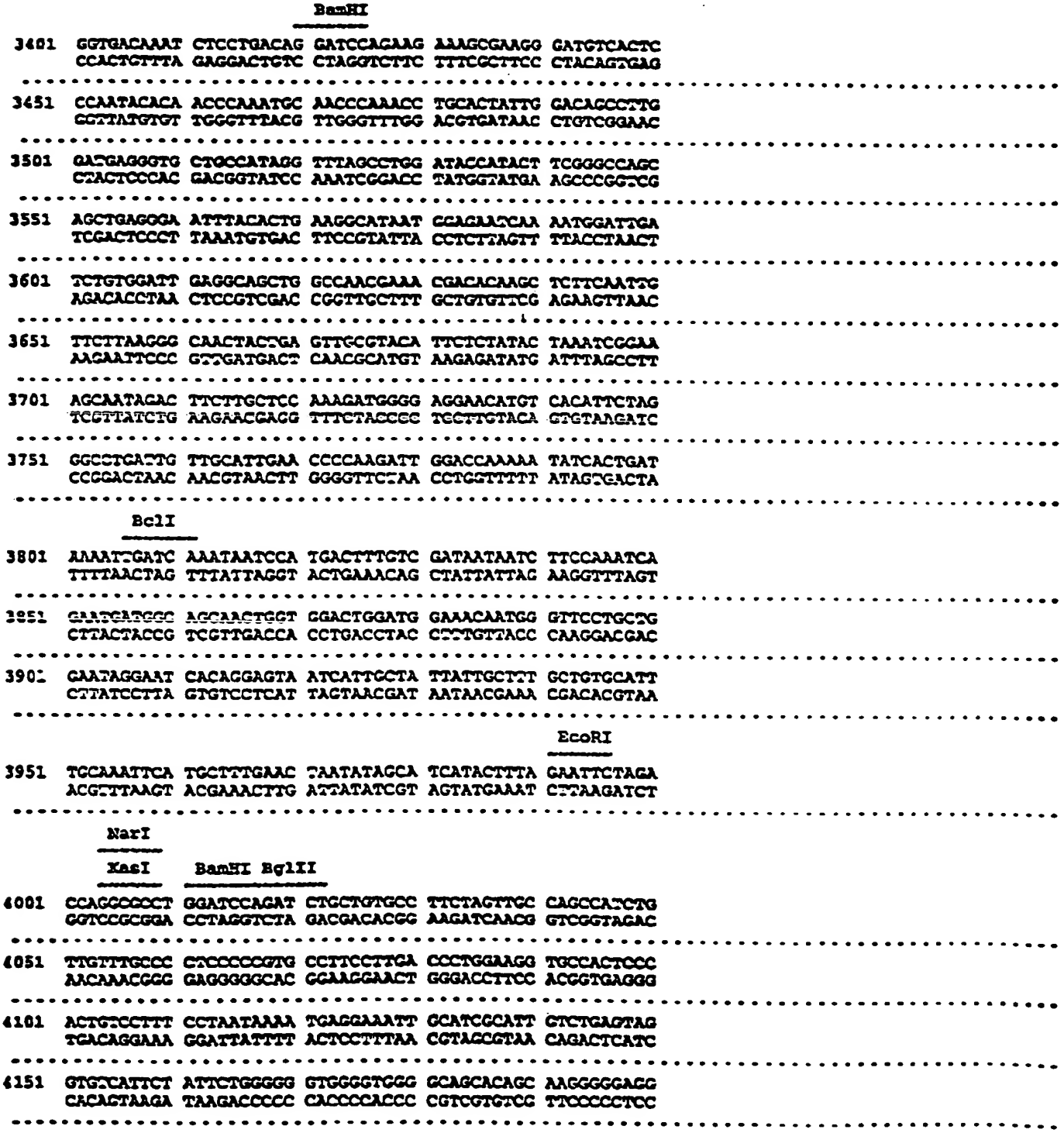
3151 CTGCCAAGAC CACCAGCCAA CCAACCAACA GCACAGAATC GACGACACTA
GACGGTTCTG GTGGTCGGTT GGTTGGTTGT CGTGTCTTAG CTGCTGTGAT

3201 AACCCAACAT CAGAGCCCTC CAGTAGAGGC ACGGGACCAT CCAGCCCCAC
TTGGGTTGTA GTCTCGGGAG GTCATCTCCG TGCCCTGGTA GGTCGGGGTG

3251 GGTCCCCAAC ACCACAGAAA GCCACGCCGA ACTTGGCAAG ACAACCCCAA
CCAGGGGTTG TGGTGTCTTT CGGTGCGGCT TGAACCGTTC TGTTGGGGTT

3301 CCACACTCCC AGAACAGCAC ACTGCCGCCA GTGCCATTCC AAGAGCCGTG
GGTGTGAGGG TCTTGTCGTG TGACGGCGGT CACGGTAAGG TTCTCGGCAC

3351 CACCCCGACG AACTCAGTGG ACCTGGCTTC CTGACGAACA CAATACGGGG
GTGGGGCTGC TTGAGTCACC TGGACCGAAG GACTGCTTGT GTTATGCCCC


BamHI
3401 GGTGACAAAT CTCCTGACAG GATCCAGAAG AAAGCGAAGG GATGTCACTC
CCACTGTTTA GAGGACTGTC CTAGGTCTTC TTTCGCTTCC CTACAGTGAG
3451 CCAATACACA ACCCAAATGC AACCCAAACC TGCACTATTG GACAGCCTTG
GGTTATGTGT TGGGTTTACG TTGGGTTTGG ACGTGATAAC CTGTCGGAAC
3501 GATGAGGGTG CTGCCATAGG TTTAGCCTGG ATACCATACT TCGGGCCAGC
CTACTCCCAC GACGGTATCC AAATCGGACC TATGGTATGA AGCCCGGTCG
3551 AGCTGAGGGA ATTTACACTG AAGGCATAAT CGAGAATCAA AATGGATTGA
TCGACTCCCT TAAATGTGAC TTCCGTATTA CCTCTTAGTT TTACCTAACT
3601 TCTGTGGATT GAGGCAGCTG GCCAACGAAA CGACACAAGC TCTTCAATTG
AGACACCTAA CTCCGTCGAC CGGTTGCTTT GCTGTGTTCG AGAAGTTAAC
3651 TTCTTAAGGG CAACTACTGA GTTGCGTACA TTCTCTATAC TAAATCGGAA
AAGAATTCCC GTTGATGACT CAACGCATGT AAGAGATATG ATTTAGCCTT
3701 AGCAATAGAC TTCTTGCTCC AAAGATGGGG AGGAACATGT CACATTCTAG
TCGTTATCTG AAGAACGAGG TTTCTACCCC TCCTTGTACA GTGTAAGATC
3751 GGCCTGATTG TTGCATTGAA CCCCAAGATT GGACCAAAAA TATCACTGAT
CCGGACTAAC AACGTAACTT GGGGTTCTAA CCTGGTTTTT ATAGTGACTA
BclI
3801 AAAATTGATC AAATAATCCA TGACTTTGTC GATAATAATC TTCCAAATCA
TTTTAACTAG TTTATTAGGT ACTGAAACAG CTATTATTAG AAGGTTTAGT
3851 GAATGATGGC AGCAACTGGT GGACTGGATG GAAACAATGG GTTCCTGCTG
CTTACTACCG TCGTTGACCA CCTGACCTAC CTTTGTTACC CAAGGACGAC
3901 GAATAGGAAT CACACGGAGTA ATCATTGCTA TTATTGCTTT GCTGTGCATT
CTTATCCTTA GTGTCCTCAT TAGTAACGAT AATAACGAAA CGACACGTAA
EcoRI
3951 TGCAAATTCA TGCTTTGAAC TAATATAGCA TCATACTTTA GAATTCTAGA
ACGTTTAAGT ACGAAACTTG ATTATATCGT AGTATGAAAT CTTAAGATCT
NarI
XasI BamHI BglII
4001 CCAGGCGCCT GGATCCAGAT CTGCTGTGCC TTCTAGTTGC CAGCCATCTG
GGTCCGCGGA CCTAGGTCTA GACGACACGG AAGATCAACG GTCGGTAGAC
4051 TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC
AACAAACGGG GAGGGGGCAC GGAAGGAACT GGGACCTTCC ACGGTGAGGG
4101 ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG
TGACAGGAAA GGATTATTTT ACTCCTTTAA CGTAGCGTAA CAGACTCATC
4151 GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGCACAGC AAGGGGGAGG
CACAGTAAGA TAAGACCCCC CACCCCACCC CGTCGTGTCG TTCCCCCTCC

SphI KpnI

```
6201  ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGGT
      TAACCCTTCT GTTATCGTCC GTACGACCCC TACGCCACCC GAGATACCCA
```

KpnI

```
4251  ACCCAGGTGC TGAAGAATTG ACCCGGTTCC TCCTGGGCCA GAAAGAAGCA
      TGGGTCCACG ACTTCTTAAC TGGGCCAAGG AGGACCCGGT CTTTCTTCGT
```

```
4301  GGCACATCCC CTTCTCTGTG ACACACCCTG TCCACGCCCC TGGTTCTTAG
      CCGTGTAGGG GAAGAGACAC TGTGTGGGAC AGGTGCGGGG ACCAAGAATC
```

```
4351  TTCCAGCCCC ACTCATAGGA CACTCATAGC TCAGGAGGGC TCCGCCTTCA
      AAGGTCGGGG TGAGTATCCT GTGAGTATCG AGTCCTCCCG AGGCGGAAGT
```

```
4401  ATCCCCACCCG CTAAAGTACT TGGAGCGGTC TCTCCCTCCC TCATCAGCCC
      TAGGGTGGGC GATTTCATGA ACCTCGCCAG AGAGGGAGGG AGTAGTCGGG
```

```
4451  ACCAAACCAA ACCTAGCCTC CAAGAGTGGG AAGAAATTAA AGCAAGATAG
      TGGTTTGGTT TGGATCGGAG GTTCTCACCC TTCTTTAATT TCGTTCTATC
```

```
4501  GCTATTAAGT GCAGAGGGAG AGAAAATGCC TCCAACATGT GAGGAAGTAA
      CGATAATTCA CGTCTCCCTC TCTTTTACGG AGGTTGTACA CTCCTTCATT
```

XmnI

```
4551  TGAGAGAAAT CATAGAATTT CTTCCGCTTC CTCGCTCACT GACTCGCTGC
      ACTCTCTTTA GTATCTTAAA GAAGGCGAAG GAGCGAGTGA CTGAGCGACG
```

```
4601  CCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA
      CGAGCCAGCA AGCCGACGCC GCTCGCCATA GTCGAGTGAG TTTCCGCCAT
```

```
4651  ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC
      TATGCCAATA GGTGTCTTAG TCCCCTATTG CGTCCTTTCT TGTACACTCG
```

```
4701  AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
      TTTTCCGGTC GTTTTCCGGT CCTTGGCATT TTTCCGGCGC AACGACCGCA
```

```
4751  TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA
      AAAAGGTATC CGAGGCGGGG GGACTGCTCG TAGTGTTTTT AGCTGCGAGT
```

```
4801  AGTCAGAGGT GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC
      TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT ATTTCTATGG TCCGCAAAGG
```

```
4851  CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG
      GGGACCTTCG AGGGAGCACG CGAGAGGACA AGGCTGGGAC GGCGAATGGC
```

```
4901  GATACCTGTC CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC
      CTATGGACAG GCGGAAAGAG GGAAGCCCTT CGCACCGCGA AAGAGTTACG
```

```
4951  TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG
      AGTGCGACAT CCATAGAGTC AAGCCACATC CAGCAAGCGA GGTTCGACCC
```

```
5001  CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
      GACACACGTG CTTGGGGGGC AAGTCGGGCT GGCGACGCGG AATAGGCCAT
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5051  ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA
      TGATAGCAGA ACTCAGGTTG GGCCATTCTG TGCTGAATAG CGGTGACCGT
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5101  GCAGCCACTG GTAACAGGAT TAGCAGACCG AGGTATGTAG GCGGTGCTAC
      CGTCGGTGAC CATTGTCCTA ATCGTCTCGC TCCATACATC CGCCACGATG
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5151  AGAGTTCTTG AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT
      TCTCAAGAAC TTCACCACCG GATTGATGCC GATGTGATCT TCCTGTCATA
.....
5201  TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT
      AACCATAGAC GCGAGACGAC TTCGGTCAAT GGAAGCCTTT TTCTCAACCA
.....
5251  AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT
      TCGAGAACTA GGCCGTTTGT TTGGTGGCGA CCATCGCCAC CAAAAAAACA
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5301  TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
      AACGTTCGTC GTCTAATGCG CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA
.....
5351  TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA
      ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA CCTTGCTTTT GAGTGCAATT
.....
5401  GGGATTTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT
      CCCTAAAACC AGTACTCTAA TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA
.....
5451  AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT
      TTTAATTTTT ACTTCAAAAT TTAGTTAGAT TTCATATATA CTCATTTGAA
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5501  GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC
      CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA GAGTCGCTAG
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5551  TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCGGGGGG GGGGGGCGCT
      ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGCCCCCC CCCCCCGCGA
.....
5601  GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG ACTCATACCA GGCCTGAATC
      CTCCAGACGG AGCACTTCTT CCACAACGAC TGAGTATGGT CCGGACTTAG
.....
5651  GCCCCATCAT CCAGCCAGAA AGTGAGGGAG CCACGGTTGA TGAGAGCTTT
      CGGGGTAGTA GGTCGGTCTT TCACTCCCTC GGTGCCAACT ACTCTCGAAA
.....
5701  GTTGTAGGTG GACCAGTTGG TGATTTTGAA CTTTTGCTTT GCCACGGAAC
      CAACATCCAC CTGGTCAACC ACTAAAACTT GAAAACGAAA CGGTGCCTTG
.....
5751  GGTCTGCGTT GTCGGGAAGA TGCGTGATCT GATCCTTCAA CTCAGCAAAA
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5801  GTTCGATTTA TTCAACAAAG CCGCCGTCCC GTCAAGTCAG CGTAATGCTC
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5851  TGCCAGTGTT ACAACCAATT AACCAATTCT GATTAGAAAA ACTCATCGAG
      ACGGTCACAA TGTTGGTTAA TTGGTTAAGA CTAATCTTTT TGAGTAGCTC
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5901  CATCAAATGA AACTGCAATT TATTCATATC AGGATTATCA ATACCATATT
      GTAGTTTACT TTGACGTTAA ATAAGTATAG TCCTAATAGT TATGGTATAA
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5951  TTTGAAAAAG CCGTTTCTGT AATGAAGGAG AAAACTCACC GAGGCAGTTC
      AAACTTTTTC GGCAAAGACA TTACTTCCTC TTTTGAGTGG CTCCGTCAAG
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6001  CATAGGATGG CAAGATCCTG GTATCGGTCT GCGATTCCGA CTCGTCCAAC
      GTATCCTACC GTTCTAGGAC CATAGCCAGA CGCTAAGGCT GAGCAGGTTG

6051  ATCAATACAA CCTATTAATT TCCCCTCGTC AAAAATAAGG TTATCAAGTG
      TAGTTATGTT GGATAATTAA AGGGGAGCAG TTTTTATTCC AATAGTTCAC

                                                    HindIII
                                                    ========
6101  AGAAATCACC ATGAGTGACG ACTGAATCCG GTGAGAATGG CAAAAGCTTA
      TCTTTAGTGG TACTCACTGC TGACTTAGGC CACTCTTACC GTTTTCGAAT

6151  TGCATTTCTT TCCAGACTTG TTCAACAGGC CAGCCATTAC GCTCGTCATC
      ACGTAAAGAA AGGTCTGAAC AAGTTGTCCG GTCGGTAATG CGAGCAGTAG

6201  AAAATCACTC GCATCAACCA AACCGTTATT CATTCGTGAT TGCGCCTGAG
      TTTTAGTGAG CGTAGTTGGT TTGGCAATAA GTAAGCACTA ACGCGGACTC

                      PvuI
                      ====
6251  CCAGACGAAA TACGCGATCG CTGTTAAAAG GACAATTACA AACAGGAATC
      GCTCTGCTTT ATGCGCTAGC GACAATTTTC CTGTTAATGT TTGTCCTTAG

6301  GAATGCAACC GGCGCAGGAA CACTGCCAGC GCATCAACAA TATTTTCACC
      CTTACGTTGG CCGCGTCCTT GTGACGGTCG CGTAGTTGTT ATAAAAGTGG

6351  TGAATCAGGA TATTCTTCTA ATACCTGGAA TGCTGTTTTC CCGGGGATCG
      ACTTAGTCCT ATAAGAAGAT TATGGACCTT ACGACAAAAG GGCCCCTAGC

6401  CAGTGGTGAG TAACCATGCA TCATCAGGAG TACGGATAAA ATGCTTGATG
      GTCACCACTC ATTGGTACGT AGTAGTCCTC ATGCCTATTT TACGAACTAC

6451  GTCCGAAGAG GCATAAATTC CGTCAGCCAG TTTAGTCTGA CCATCTCATC
      CAGCCTTCTC CGTATTTAAG GCAGTCGGTC AAATCAGACT GGTAGAGTAG

6501  TGTAACATCA TTGGCAACGC TACCTTTGCC ATGTTTCAGA AACAACTCTG
      ACATTGTAGT AACCGTTGCG ATGGAAACGG TACAAAGTCT TTGTTGAGAC

                            ClaI
                            ====
6551  GCGCATCGGG CTTCCCATAC AATCGATAGA TTGTCGCACC TGATTGCCCG
      CGCGTAGCCC GAAGGGTATG TTAGCTATCT AACAGCGTGG ACTAACGGGC

6601  ACATTATCGC GAGCCCATTT ATACCCATAT AAATCAGCAT CCATGTTGGA
      TGTAATAGCG CTCGGGTAAA TATGGGTATA TTTAGTCGTA GGTACAACCT

                      XhoI
                      ====
6651  ATTTAATCGC GGCCTCGAGC AAGACGTTTC CCGTTGAATA TGGCTCATAA
      TAAATTAGCG CCGGAGCTCG TTCTGCAAAG GGCAACTTAT ACCGAGTATT

6701  CACCCCTCGT ATTACTGTTT ATGTAAGCAG ACAGTTTTAT TGTTCATGAT
      GTGGGGAGCA TAATGACAAA TACATTCGTC TGTCAAAATA ACAAGTACTA

                                                    DraIII
                                                    ======
6751  GATATATTTT TATCTTGTGC AATGTAACAT CAGAGATTTT GAGACACAAC
      CTATATAAAA ATAGAACACG TTACATTGTA GTCTCTAAAA CTCTGTGTTG
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DraIII

6801 GTGGCTTTCC CCCCCCCCCC ATTATTGAAG CATTTATCAG GGTTATTGTC
CACCGAAAGG GGGGGGGGGG TAATAACTTC GTAAATAGTC CCAATAACAG

6851 TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
AGTACTCGCC TATGTATAAA CTTACATAAA TCTTTTTATT TGTTTATCCC

6901 GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT
CAAGGCGCGT GTAAACGGGC TTTTCACGGT GGACTGCAGA TTCTTTGGTA

6951 TACTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTTTC
ATAATAGTAC TGTAATTGGA TATTTTTATC CGCATAGTGC TCCGGGAAAG

7001 GTC
CAG

pVR 1012-GP(S)

General Description

DNA pVR 1012-GP(S)
Local object
Created: 09/14/98 03:58PM
Last Modification Date: ? (no data)
length: 7073 bp
storage type: Basic
form: Circular
Comments

Restriction Map

BalI: 1 site TGGCCA / ACCGGT

BclI: 1 site TGATCA / ACTAGT

ClaI: 1 site ATCGAT / TAGCTA

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

KasI: 1 site GGCGCC / CCGCGG

KpnI: 1 site GGTACC / CCATGG

NarI: 1 site GGCGCC / CCGCGG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

SalI: 1 site GTCGAC / CAGCTG

XbaI: 1 site TCTAGA / AGATCT

XmnI: 1 site GAANNNNTTC / CTTNNNNAAG

NdeI: 2 sites CATATG / GTATAC

EcoRV: 3 sites GATATC / CTATAG

SphI: 3 sites GCATGC / CGTACG

NcoI: 4 sites CCATGG / GGTACC

BamHI: 6 sites GGATCC / CCTAGG

Functional Map

CDS (4 signals)

CMV IE 5' UT

Start: 886 End: 1129

CMV IE INT

Start: 1130 End: 1840

TbGH

Start: 4090 End: 4642

Kan r

Start: 6138 End: 6760 (Complementary)

Misc_feature (2 signals)

CMV enhancer

Start: 248 End: 885

GP(S)

Start: 1870 End: 4089

Annotations

```
  1 TCCCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
    AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

 51 GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
    CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101 TCAGGGCCCG TCAGCGGGTG TTGCCGGGTG TCGGGGCTGG CTTAACTATG
    AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                          NdeI
151 CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
    GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

                                                 BalI
201 CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
    GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251 TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
    AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
    AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351 AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
    TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401 ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
    CGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451 CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
    GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501 CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
    GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                          NdeI
551 GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
    CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
    ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                 NcoI
651 ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
    TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

    NcoI
    -
701 GTGATCCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
    CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751 ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
    TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA
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 801  CGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
      CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
 851  TTGACGCAAA TCGGCGGTAG CCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
                                          SacII
                                          ------
 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
                                          SphI
                                          ----
1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
1251  GAACTATCCC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      CTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
1301  ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
1401  CCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
1601  TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
1651  CCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
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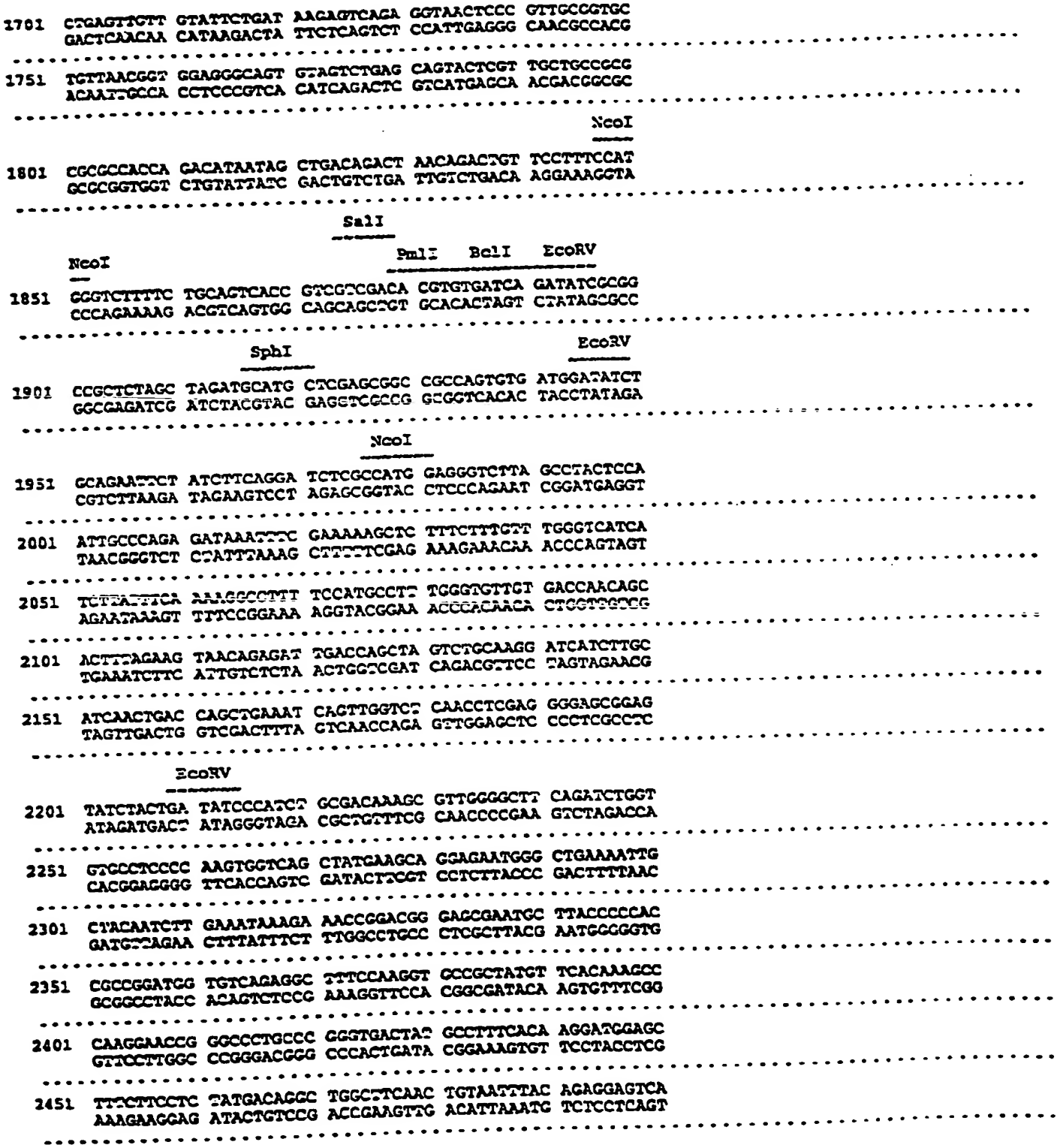
NcoI
SalI
NcoI
PmlI BclI EcoRV
SphI
EcoRV
NcoI
EcoRV

```
2501  ATTTTCCTGA GCGGGTAATC GCATTCTTGA TATTGGCTAA ACCAAAGGAA
      TAAAACGACT CCCCCATTAG CGTAAGAACT ATAACCGATT TGGTTTCCTT
.....
2551  ACGTTCCTTC AATCACCCCC CATTCGAGAG GCAGCAAACT ACACTGAAAA
      TGCAAGGAAG TTAGTGGGGG GTAAGCTCTC CGTCGTTTGA TGTGACTTTT
.....
2601  TACATCAAGT TACTATGCCA CATCCTACTT GGAGTACGAA ATCGAAAATT
      ATGTAGTTCA ATGATACGGT GTAGGATGAA CCTCATGCTT TAGCTTTTAA
.....
2651  TTGGTGCTCA ACACTCCACG ACCCTTTTCA AAATTAACAA TAATACTTTT
      AACCACCAGT TGTGAGGTGC TGGGAAAAGT TTTAATTGTT ATTATGAAAA
.....
2701  GTTCTTCTGG ACAGGCCCCA CACGCCTCAG TTCCTTTTCC AGCTGAATGA
      CAAGAAGACC TGTCCGGGGT GTGCGGAGTC AAGGAAAAGG TCGACTTACT
.....
2751  TACCATTCAA CTTCACCAAC AGTTGAGCAA CACAACTGGG AAACTAATTT
      ATGGTAAGTT GAAGTGGTTG TCAACTCGTT GTGTTGACCC TTTGATTAAA
.....
2801  GGACACTAGA TGCTAATATC AATGCTGATA TTGGTGAATG GGCTTTTTGG
      CCTGTGATCT ACGATTATAG TTACGACTAT AACCACTTAC CCGAAAAACC
.....
2851  GAAAATAAAA AAATCTCTCC GAACAACTAC CTGGAGAAGA GCTGTCTTTC
      CTTTTATTTT TTTAGAGAGG CTTGTTGATG CACCTCTTCT CGACAGAAAG
.....
2901  GAAACTTTAT CGCTCAACGA GACAGAAGAC CATGATGCGA CATCGTCGAG
      CTTTGAAATA GCGAGTTGCT CTGTCTTCTG CTACTACGCT GTAGCAGCTC
.....
2951  AACTACAAAG GGAAGAATCT CCGACCGGGC CACCAGGAAG TATTCGGACC
      TTGATGTTTC CCTTCTTAGA GGCTGGCCCG GTGGTCCTTC ATAAGCCTGG
.....
3001  TGGTTCCAAA GGATTCCCCT GGGATGGTTT CATTGCACGT ACCAGAAGGG
      ACCAAGGTTT CCTAAGGGGA CCCTACCAAA GTAACGTGCA TGGTCTTCCC
.....
3051  GAAACAACAT TGCCGTCTCA GAATTCGACA GAAGGTCGAA GAGTAGATGT
      CTTTGTTGTA ACGGCAGAGT CTTAAGCTGT CTTCCAGCTT CTCATCTACA
.....
3101  GAATACTCAG GAAACTATCA CAGAGACAAC TGCAACAATC ATAGGCACTA
      CTTATGAGTC CTTTGATAGT GTCTCTGTTG ACGTTGTTAG TATCCGTGAT
.....
3151  ACGGTAACAA CATGCAGATC TCCACCATCG GGACAGGACT GAGCTCCAGC
      TGCCATTGTT GTACGTCTAG AGGTGGTAGC CCTGTCCTGA CTCGAGGTCG
.....
                          NcoI
                          ------
3201  CAAATCCTGA GTTCCTCACC GACCATGGCA CCAAGCCCTG AGACTCAGAC
      GTTTAGGACT CAAGGAGTGG CTGGTACCGT GGTTCGGGAC TCTGAGTCTG
.....
3251  CTCCACAACC TACACACCAA AACTACCAGT GATGACCACC GAGGAACCAA
      GAGGTGTTGG ATGTGTGGTT TTGATGGTCA CTACTGGTGG CTCCTTGGTT
.....
3301  CAACACCACC GAGAAACTCT CCTGGCTCAA CAACAGAAGC ACCCACTCTC
      GTTGTGGTGG CTCTTTGAGA GGACCGAGTT GTTGTCTTCG TGGGTGAGAG
.....
3351  ACCACCCCAG AGAATATAAC AACACCGGTT AAAACTGTTT GGGCACAAGA
      TGGTGGGGTC TCTTATATTG TTGTCGCCAA TTTTGACAAA CCCGTGTTCT
.....
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3401  GTCCACAAGC AACGGTCTAA TAACTTCAAC AGTAACAGGT ATTCTTGCGA
      CAGGTGTTCG TTGCCAGATT ATTGAAGTTG TCATTGTCCA TAAGAACCCT
.....

3451  GCCTTGGACT TCGAAAACGC AGCAGAAGAC AAGTTAACAC CAGGGCCACG
      CGGAACCTGA AGCTTTTGCG TCGTCTTCTG TTCAATTGTG GTCCCGGTGC
.....

3501  GGTAAATGCA ATCCCAACTT ACACTACTGG ACTCCACAAG AACAACATAA
      CCATTTACGT TAGGGTTGAA TGTGATGACC TGACGTGTTC TTGTTGTATT
.....

                        BamHI
                       ------

3551  TGCTGCTGGG ATTGCCTGGA TCCCGTACTT TCGACCGGGT GCAGAAGGCA
      ACGACGACCC TAACGGACCT AGGGCATGAA AGCTGGCCCA CGTCTTCCGT
.....

3601  TATACACTGA AGGCCTTATG CACAACCAAA ATGCCTTAGT CTGTGGACTC
      ATATGTGACT TCCGGAATAC GTGTTGGTTT TACGGAATCA GACACCTGAG
.....

3651  AGACAACTTG CAAATGAAAC AACTCAAGCT CTCCAGCTTT TCTTAAGGGC
      TCTGTTGAAC GTTTACTTTG TTGAGTTCGA GACGTCGAAA AGAATTCCCG
.....

3701  CACGACGGAG CTGCCGACAT ATACCATACT CAATAGGAAG GCCATAGATT
      GTGCTGCCTC GACGCCTGTA TATGGTATGA GTTATCCTTC CGGTATCTAA
.....

                                      BamHI
                                     ------

3751  TCCTTCTGCG ACGATGGGGC GGGACATGTA GGATCCTGGG ACCAGATTGT
      AGGAAGACGC TGCTACCCCG CCCTGTACAT CCTAGGACCC TGGTCTAACA
.....

3801  TGCATTGAGC CACATGATTG GACCAAAAAC ATCACTGATA AAATCAACCA
      ACGTAACTCG GTGTACTAAC CTGGTTTTTG TAGTGACTAT TTTAGTTGGT
.....

3851  AATCATCCAT GATTTCATCG ACAACCCTTT ACCCAATCAG GATAATGATG
      TTAGTAGGTA CTAAAGTAGC TGTTGGGAAA TGGGTTAGTC CTATTACTAC
.....

                                   BamHI
                                  ------

3901  ATAATTGGTG GACGGGCTGG AGACAGTGGA TCCCTGCAGG AATAGGCATT
      TATTAACCAC CTGCCCGACC TCTGTCACCT AGGGACGTCC TTATCCGTAA
.....

3951  ACTGGAATTA TTATTGCAAT CATTGCTCTT CTTTGCGTCT GCAAGCTGCT
      TGACCTTAAT AATAACGTTA GTAACGAGAA GAAACGCAGA CGTTCGACGA
.....

                                                    BamHI
                                                   ------

4001  TTGTTGAATA TCAGAATTCC AGCACTGGCG GCCGTTACTA GTGGATCCGA
      AACAACTTAT AGTCTTAAGG TCGTGACCGC CGGCAATGAT CACCTAGGCT
.....

                                  NarI
                                 ------

          BamHI       XbaI       KasI     BamHI
          ------      ------     ------   ------

4051  GCTCGGATCC AAGCTCTAGA CCAGGCGCCT GGATCCAGAT CTGCTGTGCC
      CGAGCCTAGG TTCGAGATCT GGTCCGCGGA CCTAGGTCTA GACGACACGG
.....

4101  TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA
      AAGATCAACG GTCGGTAGAC AACAAACGGG GAGGGGGCAC GGAAGGAACT
.....

4151  CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA TGAGGAAATT
      GGGACCTTCC ACGGTGAGGG TGACAGGAAA GGATTATTTT ACTCCTTTAA
.....
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4201  GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG
      CGTAGCGTAA CAGACTCATC CACAGTAAGA TAAGACCCCC CACCCCACCC

                                                  SphI
4251  GCAGCACAGC AAGGCGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG
      CGTCGTGTCG TTCCCCCTCC TAACCCTTCT GTTATCGTCC GTACGACCCC

                      KpnI
4301  ATGCGGTGGG CTCTATGGGT ACCCAGGTGC TGAAGAATTG ACCCGGTTCC
      TACGCCACCC GAGATACCCA TGGGTCCACG ACTTCTTAAC TGGGCCAAGG

4351  TCCTGGGCCA GAAAGAAGCA GGCACATCCC CTTCTCTGTG ACACACCCTG
      AGGACCCGGT CTTTCTTCGT CCGTGTAGGG GAAGAGACAC TGTGTGGGAC

4401  TCCACGCCCC TGGTTCTTAG TTCCAGCCCC ACTCATAGGA CACTCATAGC
      AGGTGCGGGG ACCAAGAATC AAGGTCGGGG TGAGTATCCT GTGAGTATCG

4451  TCAGGAGGGC TCCGCCTTCA ATCCCACCCG CTAAAGTACT TGGAGCGGTC
      AGTCCTCCCG AGGCGGAAGT TAGGGTGGGC GATTTCATGA ACCTCGCCAG

4501  TCTCCCTCCC TCATCAGCCC ACCAAACCAA ACCTAGCCTC CAAGAGTGGG
      AGAGGGAGGG AGTAGTCGGG TGGTTTGGTT TGGATCGGAG GTTCTCACCC

4551  AAGAAATTAA AGCAAGATAG GCTATTAAGT GCAGAGGGAG AGAAAATGCC
      TTCTTTAATT TCGTTCTATC CGATAATTCA CGTCTCCCTC TCTTTTACGG

                                                  XmnI
4601  TCCAACATGT GAGGAAGTAA TGAGAGAAAT CATAGAATTT CTTCCGCCTC
      AGGTTGTACA CTCCTTCATT ACTCTCTTTA GTATCTTAAA GAAGGCGAAG

4651  CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
      GAGCGAGTGA CTGAGCGACG CGAGCCAGCA AGCCGACGCC GCTCGCCATA

4701  CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC
      GTCGAGTGAG TTTCCGCCAT TATGCCAATA GGTGTCTTAG TCCCCTATTG

4751  GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA
      CGTCCTTTCT TGTACACTCG TTTTCCGGTC GTTTCCGGT CCTTGGCATT

4801  AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC
      TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG GGACTGCTCG

4851  ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA
      TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT

4901  TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT
      ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG CGAGAGGACA

4951  TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
      AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG GGAAGCCCTT

5001  GCGTGGCGCT TTCTCAATGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG
      CGCACCGCGA AAGAGTTACG AGTGCGACAT CCATAGAGTC AAGCCACATC
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5051  GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA
      CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC AAGTCGGGCT
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5101  CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC
      GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG GGCCATTCTG
.....
5151  ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG
      TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA ATCGTCTCGC
.....
5201  AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACTACGG
      TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG GATTGATGCC
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5251  CTACACCAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
      GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC TTCGGTCAAT
.....
5301  CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT
      GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT TTGGTGGCGA
.....
5351  GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA
      CCATCGCCAC CAAAAAAACA AACGTTCGTC GTCTAATGCG CGTCTTTTTT
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5401  ACGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT
      TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA
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5451  GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG
      CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA TAGTTTTTCC
.....
5501  ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA
      TAGAAGTGGA TCTAGGAAAA TTTAATTTTT ACTTCAAAAT TTAGTCAGAT
.....
5551  AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
      TTCATATATA CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC
.....
5601  AGGCACCTAT CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA
      TCCGTGGATA GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT
.....
5651  CTCCGGGGGG GGGGGGCGCT GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG
      GAGGCCCCCC CCCCCCGCGA CTCCAGACGG AGCACTTCTT CCACAACGAC
.....
5701  ACTCATACCA GGCCTGAATC GCCCCATCAT CCAGCCAGAA AGTGAGGGAG
      TGAGTATGGT CCGGACTTAG CGGGGTAGTA GGTCGGTCTT TCACTCCCTC
.....
5751  CCACGGTTGA TGAGAGCTTT GTTGTAGGTG GACCAGTTGG TGATTTTGAA
      GGTGCCAACT ACTCTCGAAA CAACATCCAC CTGGTCAACC ACTAAAACTT
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5801  CTTTTGCTTT GCCACGGAAC GGTCTGCGTT GTCGGGAAGA TGCGTGATCT
      GAAAACGAAA CGGTGCCTTG CCAGACGCAA CAGCCCTTCT ACGCACTAGA
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5851  GATCCTTCAA CTCAGCAAAA GTTCGATTTA TTCAACAAAG CCGCCGTCCC
      CTAGGAAGTT GAGTCGTTTT CAAGCTAAAT AAGTTGTTTC GGCGGCAGGG
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5901  GTCAAGTCAG CGTAATGCTC TGCCAGTGTT ACAACCAATT AACCAATTCT
      CAGTTCAGTC GCATTACGAG ACGGTCACAA TGTTGGTTAA TTGGTTAAGA
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5951  GATTAGAAAA ACTCATCGAG CATCAAATGA AACTGCAATT TATTCATATC
      CTAATCTTTT TGAGTAGCTC GTAGTTTACT TTGACGTTAA ATAAGTATAG
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6001  AGGATTATCA ATACCATATT TTTGAAAAAG CCGTTTCCGT AATGAAGGAG
      TCCTAATAGT TATGGTATAA AAACTTTTTC GGCAAAGACA TTACTTCCTC
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6051  AAAACTCACC GAGGCAGTTC CATACGATGG CAAGATCCTG GTATCGGTCT
      TTTTGAGTGG CTCCGTCAAG GTATCCTACC GTTCTAGGAC CATAGCCAGA
.....

6101  GCGATTCCGA CTCGTCCAAC ATCAATACAA CCTATTAATT TCCCCTCGTC
      CGCTAAGGCT GAGCAGGTTG TAGTTATGTT CGATAATTAA AGGGGAGCAG
.....

6151  AAAAATAAGG TTATCAAGTG AGAAATCACC ATGAGTGACG ACTGAATCCG
      TTTTTATTCC AATAGTTCAC TCTTTAGTGG TACTCACTGC TGACTTAGGC
.....

                  HindIII
                  ═══════
6201  GTGAGAATGG CAAAAGCTTA TGCATTTCTT TCCAGACTTG TTCAACAGGC
      CACTCTTACC GTTTTCGAAT ACGTAAAGAA AGGTCTGAAC AAGTTGTCCG
.....

6251  CAGCCATTAC GCTCGTCATC AAAATCACTC GCATCAACCA AACCGTTATT
      GTCGGTAATG CGAGCAGTAG TTTTAGTGAG CGTAGTTGGT TTGGCAATAA
.....

                                        PvuI
                                        ════
6301  CATTCGTGAT TCCGCCTGAG CGAGACGAAA TACGCGATCG CTGTTAAAAG
      GTAAGCACTA ACGCGGACTC GCTCTGCTTT ATGCGCTAGC GACAATTTTC
.....

6351  GACAATTACA AACAGGAATC GAATGCAACC GGCGCAGGAA CACTGCCAGC
      CTGTTAATGT TTGTCCTTAG CTTACGTTGG CCGCGTCCTT GTGACGGTCG
.....

6401  GCATCAACAA TATTTTCACC TGAATCAGGA TATTCTTCTA ATACCTGGAA
      CGTAGTTGTT ATAAAAGTGG ACTTAGTCCT ATAAGAAGAT TATGGACCTT
.....

6451  TGCTGTTTTC CCGGGGATCG CAGTGGTGAG TAACCATGCA TCATCAGGAG
      ACGACAAAAG GGCCCCTAGC GTCACCACTC ATTGGTACGT AGTAGTCCTC
.....

6501  TACGGATAAA ATGCTTGATG GTCGGAAGAG GCATAAATTC CGTCAGCCAG
      ATGCCTATTT TACGAACTAC CAGCCTTCTC CGTATTTAAG GCAGTCGGTC
.....

6551  TTTATCTCTGA CCATCTCATC TGTAACATCA TTGGCAACGC TACCTTTGCC
      AAATCAGACT GGTAGAGTAG ACATTGTAGT AACCGTTGCG ATGGAAACGG
.....

                                                  ClaI
                                                  ════
6601  ATGTTTCAGA AACAACTCTG GCGCATCGGG CTTCCCATAC AATCGATAGA
      TACAAAGTCT TTGTTGAGAC CGCGTAGCCC GAAGGGTATG TTAGCTATCT
.....

6651  TTGTCGCACC TGATTGCCCG ACATTATCGC GAGCCCATTT ATACCCATAT
      AACAGCGTGG ACTAACGGGC TGTAATAGCG CTCGGGTAAA TATGGGTATA
.....

6701  AAATCAGCAT CCATGTTGGA ATTTAATCGC GGCCTCGAGC AAGACGTTTC
      TTTAGTCGTA GGTACAACCT TAAATTAGCG CCGGAGCTCG TTCTGCAAAG
.....

6751  CCGTTGAATA TGGCTCATAA CACCCCTTGT ATTACTGTTT ATGTAAGCAG
      GGCAACTTAT ACCGAGTATT GTGGGGAACA TAATGACAAA TACATTCGTC
.....

6801  ACAGTTTTAT TGTTCATGAT GATATATTTT TATCTTGTGC AATGTAACAT
      TGTCAAAATA ACAAGTACTA CTATATAAAA ATAGAACACG TTACATTGTA
.....
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DraIII

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6851  CAGAGATTTT GAGACACAAC GTGGCTTTCC CCCCCCCCCC ATTATTGAAG
      GTCTCTAAAA CTCTGTGTTG CACCGAAAGG GGGGGGGGGG TAATAACTTC
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6901  CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT
      GTAAATAGTC CCAATAACAG AGTACTCGCC TATGTATAAA CTTACATAAA
.....
6951  AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA
      TCTTTTTATT TGTTTATCCC CAAGGCGCGT GTAAAGGGGC TTTTCACGGT
.....
7001  CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG
      GGACTGCAGA TTCTTTGGTA ATAATAGTAC TGTAATTGGA TATTTTTATC
.....
7051  GCGTATCACG AGGCCCTTTC GTC
      CGCATAGTGC TCCGGGAAAG CAG
.....
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SEQUENCE LISTING ID NO: 3

pVR 1012-GP(Z)

General Description

DNA pVR 1012-GP(Z)
Local object
Created: 09/15/98 05:06PM
Last Modification Date: ? (no data)
length: 7285 bp
storage type: Basic
form.: Circular

Comments

Restriction Map

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

HpaI: 1 site GTTAAC / CAATTG

KasI: 1 site GGCGCC / CCGCGG

NarI: 1 site GGCGCC / CCGCGG

NotI: 1 site GCGGCCGC / CGCCGGCG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

XbaI: 1 site TCTAGA / AGATCT

XhoI: 1 site CTCGAG / GAGCTC

EcoRV: 2 sites GATATC / CTATAG

NcoI: 2 sites CCATGG / GGTACC

NdeI: 2 sites CATATG / GTATAC

SphI: 2 sites GCATGC / CGTACG

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1860

TbGH
Start: 4302 End: 4854

Kan r
Start: 6350 End: 6972 (Complementary)

Misc_featur (2 signals)

CMV enhancer

Start: 248 End: 885

GP(Z)

Start: 1870 End: 4301

Annotations

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  1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
     AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

 51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
     CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101  TCAGGGCGCC TCAGCCGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
     AGTCCCGCGG AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                        NdeI
                                       ------
151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
     GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
     GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
     AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
     AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
     TGTATTGAAT CCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
     GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
     GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                         NdeI
                        ------
551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
     CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
     ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                    NcoI
                                                   ------
651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
     TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

     NcoI
     -
701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
     CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751  ACGGCGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
     TGCCGCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
     CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
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 851  TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
                                                  SacII
 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
                                                  SphI
1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
1301  ACGGACTCTC TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAG ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
1601  TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
1701  CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
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       HpaI
1751  TGTTAACGGT GGACGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC
.....
                                                      NcoI
1801  CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA
.....
      EcoO                          PmlI          EcoRVNotI
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC
.....
                   NarI
      NotI XbaI    XasI
1901  CCGCTCTAGA CCAGGCGCCT GGATCGATCC GCGATGAAGA TTAAGCCGAC
      GGCGAGATCT GGTCCGCGGA CCTAGCTAGG CGCTACTTCT AATTCGGCTG
.....
1951  AGTGAGCGTA ATCTTCATCT CTCTTAGATT ATTTGTTTTC CACAGTAGGG
      TCACTCGCAT TAGAAGTAGA GAGAATCTAA TAAACAAAAG GTCTCATCCC
.....
2001  GTCGTCAGGT CCTTTTCAAT CGTGTAACCA AAATAAACTC CACTAGAAGG
      CAGCAGTCCA GGAAAAGTTA GCACATTGGT TTTATTTGAG GTGATCTTCC
.....
2051  ATATTGTGGG GCAACAACAC AATGGGCGTT ACAGGAATAT TCCAGTTACC
      TATAACACCC CGTTGTTGTG TTACCCGCAA TGTCCTTATA AGGTCAATGG
.....
2101  TCGTGATCGA TTCAAGAGGA CATCATTCTT TCTTTGGGTA ATTATCCTTT
      AGCACTAGCT AAGTTCTCCT GTAGTAAGAA AGAAACCCAT TAATAGGAAA
.....
2151  TCCAAAGAAC ATTTTCCATC CCACTTGGAG TCATCCACAA TAGCACACTA
      AGGTTTCTTG TAAAAGGTAG GGTGAACCTC AGTAGGTGTT ATCGTGTAAT
.....
2201  CAGGTTAGTG ATGTCGACAA ACTAGTTTGT CGTGACAAAC TGTCATCCAC
      GTCCAATCAC TACAGCTGTT TGATCAAACA GCACTGTTTG ACAGTAGGTG
.....
2251  AAATCAATTG AGATCAGTTG GACTGAATCT CGAAGGGAAT GGAGTGGCAA
      TTTAGTTAAC TCTAGTCAAC CTGACTTAGA GCTTCCCTTA CCTCACCGTT
.....
2301  CTGACGTGCC ATCTGCAACT AAAAGATGGG GCTTCAGGTC CGGTGTCCCA
      GACTGCACGG TAGACGTTGA TTTTCTACCC CGAAGTCCAG GCCACAGGGT
.....
2351  CCAAAGGTGG TCAATTATGA ACCTGGTGAA TGGGCTGAAA ACTGCTACAA
      GGTTTCCACC AGTTAATACT TCGACCACTT ACCCGACTTT TGACGATGTT
.....
2401  TCTTGAAATC AAAAAACCTG ACGCGAGTGA GTGTCTACCA GCAGCGCCAG
      AGAACTTTAG TTTTTTGGAC TGCGCTCACT CACAGATGGT CGTCGCGGTC
.....
2451  ACGGGATTCG GGGCTTCCCC CGGTGCCCGT ATGTGCACAA AGTATCAGGA
      TGCCCTAAGC CCCGAAGGGG GCCACGGCCA TACACGTGTT TCATAGTCCT
.....
2501  ACGGGACCGT GTGCCCGAGA CTTTGCCTTC CATAAAGAGG GTGCTTTCTT
      TGCCCTGGCA CACGGCCTCT GAAACGGAAG GTATTTCTCC CACGAAAGAA
.....
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2551  CCTGTATGAT CGACTTGCTT CCACAGTTAT CTACCGAGGA ACGACTTTCG
      GGACATACTA GCTGAACGAA GGTGTCAATA GATGGCTCCT TGCTGAAAGC
.....
2601  CTGAAGGTGT CGTTGCATTT CTGATACTGC CCCAAGCTAA GAAGGACTTC
      GACTTCCACA GCAACGTAAA GACTATGACG GGGTTCGATT CTTCCTGAAG
.....
2651  TTCAGCTCAC ACCCCTTGAG AGAGCCGGTC AATGCAACGG AGGACCCGTC
      AAGTCGAGTG TGGGGAACTC TCTCGGCCAG TTACGTTGCC TCCTGGGCAG
.....
                                  EcoRV
                                 ------
2701  TAGTGGCTAC TATTCTACCA CAATTAGATA TCAGGCTACC GGTTTTGCAA
      ATCACCGATG ATAAGATGGT GTTAATCTAT AGTCCGATGG CCAAAACGTT
.....
2751  CCAATGAGAC AGAGTACTTG TTCGAGGTTG ACAATTTGAC CTACGTCCAA
      GGTTACTCTG TCTCATGAAC AAGCTCCAAC TGTTAAACTG GATGCAGGTT
.....
2801  CTTGAATCAA GATTCACACC ACAGTTTCTG CTCCAGCTGA ATGAGACAAT
      GAACTTAGTT CTAAGTGTGG TGTCAAAGAC GAGGTCGACT TACTCTGTTA
.....
2851  ATATACAAGT GGGAAAAGGA GCAATACCAC GGGAAAACTA ATTTGGAAGG
      TATATGTTCA CCCTTTTCCT CGTTATGGTG CCCTTTTGAT TAAACCTTCC
.....
2901  TCAACCCCGA AATTGATACA ACAATCGGGG AGTGGGCCTT CTGGGAAACT
      AGTTGGGGCT TTAACTATGT TGTTAGCCCC TCACCCGGAA GACCCTTTGA
.....
2951  AAAAAAACC TCACTAGAAA AATTCGCAGT CAAGAGTTGT CTTTCACAGT
      TTTTTTTGG AGTGATCTTT TTAAGCGTCA GTTCTCAACA GAAAGTGTCA
.....
3001  TGTATCAAAC GGAGCCAAAA ACATCAGTGG TCAGAGTCCG GCGCGAACTT
      ACATAGTTTG CCTCGGTTTT TGTAGTCACC AGTCTCAGGC CGCGCTTGAA
.....
3051  CTTCCGACCC AGGGACCAAC ACAACAACTG AAGACCACAA AATCATGGCT
      GAAGGCTGGG TCCCTGGTTG TGTTGTTGAC TTCTGGTGTT TTAGTACCGA
.....
3101  TCAGAAAATT CCTCCGCAAT GGTTCAAGTG CACAGTCAAG GAAGGGAAGC
      AGTCTTTTAA GGAGACGTTA CCAAGTTCAC GTGTCAGTTC CTTCCCTTCG
.....
3151  TGCAGTGTCG CATCTAACAA CCCTTGCCAC AATCTCCACG AGTCCCCAAT
      ACGTCACAGC GTAGATTGTT GGGAACGGTG TTAGAGGTGC TCAGGGGTTA
.....
3201  CCCTCACAAC CAAACCAGGT CCGGACAACA GCACCCATAA TACACCCGTG
      GGGAGTGTTG GTTTGGTCCA GGCCTGTTGT CGTGGGTATT ATGTGGGCAC
.....
3251  TATAAACTTG ACATCTCTGA GGCAACTCAA GTTGAACAAC ATCACCGCAG
      ATATTTGAAC TGTAGAGACT CCGTTGAGTT CAACTTGTTG TAGTGGCGTC
.....
3301  AACAGACAAC GACAGCACAG CCTCCGACAC TCCCTCTGCC ACGACCGCAG
      TTGTCTGTTG CTGTCGTGTC GGAGGCTGTG AGGGAGACGG TGCTGGCGTC
.....
3351  CCGGACCCCC AAAAGCAGAG AACACCAACA CGAGCAAGAG CACTGACTTC
      GGCCTGGGGG TTTTCGTCTC TTGTGGTTGT GCTCGTTCTC GTGACTGAAG
.....
3401  CTGGACCCCG CCACCACAAC AAGTCCCCAA AACCACAGCG AGACCGCTGG
      GACCTGGGGC GGTGGTGTTG TTCAGGGGTT TTGGTGTCGC TCTGGCGACC
.....
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3451  CAACAACAAC ACTCATCACC AAGATACCGG AGAAGAGAGT GCCAGCAGCG
      GTTGTTGTTG TGAGTAGTGG TTCTATGGCC TCTTCTCTCA CGGTCGTCGC
.....
3501  GGAAGCTAGG CTTAATTACC AATACTATTG CTGGAGTCGC ACGACTGATC
      CCTTCGATCC GAATTAATGG TTATGATAAC GACCTCAGCG TCCTGACTAG
.....
3551  ACAGGCGGGA GAAGAACTCG AAGAGAAGCA ATTGTCAATG CTCAACCCAA
      TGTCCGCCCT CTTCTTGAGC TTCTCTTCGT TAACAGTTAC GAGTTGGGTT
.....
3601  ATCCAACCCT AATTTACATT ACTGGACTAC TCAGGATGAA GGTGCTGCAA
      TAGGTTGGGA TTAAATGTAA TGACCTGATG AGTCCTACTT CCACGACGTT
.....
3651  TCGGACTGGC CTGGATACCA TATTCCGGGC CAGCAGCCGA GGGAATTTAC
      AGCCTGACCG GACCTATGGT ATAAAGCCCG GTCGTCGGCT CCCTTAAATG
.....
3701  ATAGAGGGGC TAATGCACAA TCAAGATGGT CTAATCTGTG GGTTGAGACA
      TATCTCCCCG ATTACGTGTT AGTTCTACCA AATTAGACAC CCAACTCTGT
.....
3751  GCTGGCCAAC GAGACGACTC AAGCTCTTCA ACTGTTCCTG AGAGCCACAA
      CGACCGGTTG CTCTGCTGAG TTCGAGAAGT TGACAAGGAC TCTCGGTGTT
.....
3801  CTGAGCTACG CACCTTTTCA ATCCTCAACC GTAAGGCAAT CGATTCCTTG
      GACTCGATGC GTGGAAAAGT TAGGAGTTGG CATTCCGTTA ACTAAAGAAC
.....
3851  CTGCAGCGAT GGGCCGGCAC ATGCCACATT CTGGGACCGG ACTGCTGTAT
      GACGTCGCTA CCCCGCCGTG TACGGTGTAA GACCCTGGCC TGACGACATA
.....
3901  CGAACCACAT GATTGGACCA AGAACATAAC AGACAAAATC GATCAGATTA
      GCTTGGTGTA CTAACCTGGT TCTTGTATTG TCTGTTTTAA CTAGTCTAAT
.....
3951  TTCATGATTT TGTTGATAAA ACCCTTCCGG ACCAGGGGGA CAATGACAAT
      AAGTACTAAA ACAACCTATTT TGGGAAGGCC TGGTCCCCCT GTTACTGTTA
.....
4001  TGGTGGACAG GATGGAGACA ATGGATACCG GCAGGTATTG GAGTTACAGG
      ACCACCTGTC CTACCTCTGT TACCTATGGC CGTCCATAAC CTCAATGTCC
.....
4051  CGTTATAATT GCAGTTATCG CTTTATTCTG TATATGCAAA TTTGTCTTTT
      GCAATATTAA CGTCAATAGC GAAATAAGAC ATATACGTTT AAACAGAAAA
.....
4101  AGTTTTTCTT CAGATTGCTT CATGGAAAAG CTCAGCCTCA AATCAATGAA
      TCAAAAAGAA GTCTAACGAA GTACCTTTTC GAGTCGGAGT TTAGTTACTT
.....
4151  ACCAGGATTT AATTATATGG ATTACTTGAA TCCAAGATTA CTTGACAAAT
      TGGTCCTAAA TTAATATACC TAATGAACTT AGATTCTAAT GAACTGTTTA
.....
4201  GATAATATAA TACACTGGAG CTTTAAACAT AGCCAATGTG ATTCTAACTC
      CTATTATATT ATGTGACCTC GAAATTTGTA TCGGTTACAC TAAGATTGAG
.....
4251  CTTTAAACTC ACAGTTAATC ATAAACAAGG TTTGGTACCG AGCTCGAATT
      GAAATTTGAG TGTCAATTAG TATTTGTTCC AAACCATGGC TCGAGCTTAA
.....
4301  ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG
      TAGACGACAC GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGGGC
.....
4351  TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA
      ACGGAACGAA CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT
.....
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4401  AATCAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG
      TTACTCCTTT AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC
.....

4451  GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA GACAATAGCA
      CCCACCCCAC CCCGTCGTGT CGTTCCCCCT CCTAACCCTT CTGTTATCGT
.....

        SphI
       ------
4501  GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCCAGGT GCTGAAGAAT
      CCGTACGACC CCTACGCCAC CCGAGATACC CATGGGTCCA CGACTTCTTA
.....

4551  TGACCCGGTT CCTCCTGGGC CAGAAAGAAG CAGGCACATC CCCTTCTCTG
      ACTGGGCCAA GGAGGACCCG GTCTTTCTTC GTCCGTGTAG GGGAAGAGAC
.....

4601  TGACACACCC TGTCCACGCC CCTGGTTCTT AGTTCCAGCC CCACTCATAG
      ACTGTGTGGG ACAGGTGCGG GGACCAAGAA TCAAGGTCGG GGTGAGTATC
.....

4651  GACACTCATA GCTCAGGAGG GCTCCGCCTT CAATCCCACC CGCTAAAGTA
      CTGTGAGTAT CGAGTCCTCC CGAGGCGGAA GTTAGGGTGG GCGATTTCAT
.....

4701  CTTGGAGCGG TCTCTCCCTC CCTCATCAGC CCACCAAACC AAACCTAGCC
      GAACCTCGCC AGAGAGGGAG GGAGTAGTCG GGTGGTTTGG TTTGGATCGG
.....

4751  TCCAAGAGTG GGAAGAAATT AAAGCAAGAT AGGCTATTAA GTGCAGAGGG
      AGGTTCTCAC CCTTCTTTAA TTTCGTTCTA TCCGATAATT CACGTCTCCC
.....

4801  AGAGAAAATG CCTCCAACAT GTGAGGAAGT AATGAGAGAA ATCATAGAAT
      TCTCTTTTAC GGAGGTTGTA CACTCCTTCA TTACTCTCTT TAGTATCTTA
.....

4851  TTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC
      AAGAAGGCGA AGGAGCGAGT GACTGAGCGA CGCGAGCCAG CAAGCCGACG
.....

4901  GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA
      CCGCTCGCCA TAGTCGAGTG AGTTTCCGCC ATTATGCCAA TAGGTGTCTT
.....

4951  TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
      AGTCCCCTAT TGCGTCCTTT CTTGTACACT CGTTTTCCGG TCGTTTTCCG
.....

5001  CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC
      GTCCTTGGCA TTTTTCCGGC GCAACGACCG CAAAAAGGTA TCCGAGGCGG
.....

5051  CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC
      GGGGACTGCT CGTAGTGTTT TTAGCTGCGA GTTCAGTCTC CACCGCTTTG
.....

5101  CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT
      GGCTGTCCTG ATATTTCTAT GGTCCGCAAA GGGGGACCTT CGAGGGAGCA
.....

5151  GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC
      CGCGAGAGGA CAAGGCTGGG ACGGCGAATG GCCTATGGAC AGGCGGAAAG
.....

5201  TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG TAGGTATCTC
      AGGGAAGCCC TTCGCACCGC GAAAGAGTTA CGAGTGCGAC ATCCATAGAG
.....

5251  AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
      TCAAGCCACA TCCAGCAAGC GAGGTTCGAC CCGACACACG TGCTTGGGGG
.....
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5301  CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA
      GCAAGTCGGG CTGGCGACGC GGAATAGGCC ATTGATAGCA GAACTCAGGT
.....
5351  ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG
      TGGGCCATTC TGTGCTGAAT AGCGGTGACC GTCGTCGGTG ACCATTGTCC
.....
5401  ATTAGCAGAG CGAGGTATGT AGCCGGTGCT ACAGAGTTCT TGAAGTGGTG
      TAATCGTCTC GCTCCATACA TCCGCCACGA TGTCTCAAGA ACTTCACCAC
.....
5451  GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC
      CGGATTGATG CCGATGTGAT CTTCCTGTCA TAAACCATAG ACGCGAGACG
.....
5501  TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
      ACTTCGGTCA ATGGAAGCCT TTTTCTCAAC CATCGAGAAC TAGGCCGTTT
.....
5551  CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
      GTTTGGTGGC GACCATCGCC ACCAAAAAAA CAAACGTTCG TCGTCTAATG
.....
5601  GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT
      CGCGTCTTTT TTTCCTAGAG TTCTTCTAGG AAACTAGAAA AGATGCCCCA
.....
5651  CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA
      GACTGCGAGT CACCTTGCTT TTGAGTGCAA TTCCCTAAAA CCAGTACTCT
.....
5701  TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT
      AATAGTTTTT CCTAGAAGTG GATCTAGGAA AATTTAATTT TTACTTCAAA
.....
5751  TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT
      ATTTAGTTAG ATTTCATATA TACTCATTTG AACCAGACTG TCAATGGTTA
.....
5801  GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC
      CGAATTAGTC ACTCCGTGGA TAGAGTCGCT AGACAGATAA AGCAAGTAGG
.....
5851  ATAGTTGCCT GACTCCGGGG GGGGGGGGCG CTGAGGTCTG CCTCGTGAAG
      TATCAACGGA CTGAGGCCCC CCCCCCCCGC GACTCCAGAC GGAGCACTTC
.....
5901  AAGGTGTTGC TGACTCATAC CAGGCCTGAA TCGCCCCATC ATCCAGCCAG
      TTCCACAACG ACTGAGTATG GTCCGGACTT AGCGGGGTAG TAGGTCGGTC
.....
5951  AAAGTGAGGG AGCCACGGTT GATGAGAGCT TTGTTGTAGG TGGACCAGTT
      TTTCACTCCC TCGGTGCCAA CTACTCTCGA AACAACATCC ACCTGGTCAA
.....
6001  GGTGATTTTG AACTTTTGCT TTGCCACGGA ACGGTCTGCG TTGTCGGGAA
      CCACTAAAAC TTGAAAACGA AACGGTGCCT TGCCAGACGC AACAGCCCTT
.....
6051  GATGCGTGAT CTGATCCTTC AACTCAGCAA AAGTTCGATT TATTCAACAA
      CTACGCACTA GACTAGGAAG TTGAGTCGTT TTCAAGCTAA ATAAGTTGTT
.....
6101  AGCCGCCGTC CCGTCAAGTC AGCGTAATGC TCTGCCAGTG TTACAACCAA
      TCGGCGGCAG GGCAGTTCAG TCGCATTACG AGACGGTCAC AATGTTGGTT
.....
6151  TTAACCAATT CTGATTAGAA AAACTCATCG AGCATCAAAT GAAACTGCAA
      AATTGGTTAA GACTAATCTT TTTGAGTAGC TCGTAGTTTA CTTTGACGTT
.....
6201  TTTATTCATA TCAGGATTAT CAATACCATA TTTTTGAAAA AGCCGTTTCT
      AAATAAGTAT AGTCCTAATA GTTATGGTAT AAAAACTTTT TCGGCAAAGA
.....
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6251  GTAATGAAGG AGAAAACTCA CCGAGGCAGT TCCATAGGAT GGCAAGATCC
      CATTACTTCC TCTTTTGAGT GGCTCCGTCA AGGTATCCTA CCGTTCTAGG
.....
6301  TGGTAACGGT CTGCGATTCC GACTCGTCCA ACATCAATAC AACCTATTAA
      ACCATAGCCA GACGCTAAGG CTGAGCAGGT TGTAGTTATG TTGGATAATT
.....
6351  TTTCCCCTCG TCAAAAATAA GGTTATCAAG TGAGAAATCA CCATGAGTGA
      AAAGGGGAGC AGTTTTTATT CCAATAGTTC ACTCTTTAGT GGTACTCACT
.....
                                HindIII
                                ========
6401  CGACTGAATC CGGTGAGAAT GGCAAAAGCT TATGCATTTC TTTCCAGACT
      GCTGACTTAG GCCACTCTTA CCGTTTTCGA ATACGTAAAG AAAGGTCTGA
.....
6451  TGTTCAACAG GCCAGCCATT ACGCTCGTCA TCAAAATCAC TCGCATCAAC
      ACAAGTTGTC CGGTCGGTAA TGCGAGCAGT AGTTTTAGTG AGCGTAGTTG
.....
                                                      PvuI
                                                      ====
6501  CAAACCGTTA TTCATTCGTG ATTGCGCCTG AGCGAGACGA AATACGCGAT
      GTTTGGCAAT AAGTAAGCAC TAACGCGGAC TCGCTCTGCT TTATGCGCTA
.....
      PvuI
      ==
6551  CGCTGTTAAA AGGACAATTA CAAACAGGAA TCGAATGCAA CCGGCGCAGG
      GCGACAATTT TCCTGTTAAT GTTTGTCCTT AGCTTACGTT GGCCGCGTCC
.....
6601  AACACTGCCA GCGCATCAAC AATATTTTCA CCTGAATCAG GATATTCTTC
      TTGTGACGGT CGCGTAGTTG TTATAAAAGT GGACTTAGTC CTATAAGAAG
.....
6651  TAATACCTGG AATGCTGTTT TCCCGGGGAT CGCAGTGGTG AGTAACCATG
      ATTATGGACC TTACGACAAA AGGGCCCCTA GCGTCACCAC TCATTGGTAC
.....
6701  CATCATCAGG AGTACGGATA AAATGCTTGA TGGTCGGAAG AGGCATAAAT
      GTAGTAGTCC TCATGCCTAT TTTACGAACT ACCAGCCTTC TCCGTATTTA
.....
6751  TCCGTCAGCC AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC
      AGGCAGTCGG TCAAATCAGA CTGGTAGAGT AGACATTGTA GTAACCGTTG
.....
6801  GCTACCTTTG CCATGTTTCA GAAACAACTC TGGCGCATCG GGCTTCCCAT
      CGATGGAAAC GGTACAAAGT CTTTGTTGAG ACCGCGTAGC CCGAAGGGTA
.....
6851  ACAATCGATA GATTGTCGCA CCTGATTGCC CGACATTATC GCGAGCCCAT
      TGTTAGCTAT CTAACAGCGT GGACTAACGG GCTGTAATAG CGCTCGGGTA
.....
                                                      XhoI
                                                      ====
6901  TTATACCCAT ATAAATCAGC ATCCATGTTG GAATTTAATC GCGGCCTCGA
      AATATGGGTA TATTTAGTCG TAGGTACAAC CTTAAATTAG CGCCGGAGCT
.....
      XhoI
      ====
6951  GCAAGACGTT TCCCGTTGAA TATGGCTCAT AACACCCCTT GTATTACTGT
      CGTTCTGCAA AGGGCAACTT ATACCGAGTA TTGTGGGGAA CATAATGACA
.....
7001  TTATGTAAGC AGACAGTTTT ATTGTTCATG ATGATATATT TTTATCTTGT
      AATACATTCG TCTGTCAAAA TAACAAGTAC TACTATATAA AAATAGAACA
.....
```

DraIII

```
7051  GCAATGTAAC ATCAGAGATT TTGAGACACA ACGTGGCTTT CCCCCCCCCC
      CGTTACATTG TAGTCTCTAA AACTCTGTGT TGCACCGAAA GGGGGGGGGG
.....
7101  CCATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT
      GGTAATAACT TCGTAAATAG TCCCAATAAC AGAGTACTCG CCTATGTATA
.....
7151  TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC
      AACTTACATA AATCTTTTTA TTTGTTTATC CCCAAGGCGC GTGTAAAGGG
.....
7201  CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC
      GCTTTTCACG GTGGACTGCA GATTCTTTGG TAATAATAGT ACTGTAATTG
.....
7251  CTATAAAAAT AGGCGTATCA CGAGGCCCTT TCGTC
      GATATTTTTA TCCGCATAGT GCTCCGGGAA AGCAG
.....
```

pVR 1012-SGP(Z)

General Description

DNA pVR 1012-SGP(Z)
Local object
Created: 09/14/98 04:29PM
Last Modified: 09/15/98 04:50PM
length: 7272 bp
storage type: Basic
form: Circular
Comments

Restriction Map

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

HpaI: 1 site GTTAAC / CAATTG

KpnI: 1 site GGTACC / CCATGG

NotI: 1 site GCGGCCGC / CGCCGGCG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

XbaI: 1 site TCTAGA / AGATCT

XhoI: 1 site CTCGAG / GAGCTC

EcoRV: 2 sites GATATC / CTATAG

NcoI: 2 sites CCATGG / GGTACC

NdeI: 2 sites CATATG / GTATAC

SphI: 2 sites GCATGC / CGTACG

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1840

TbGH
Start: 4289 End: 4861

Kan r
Start: 6337 End: 6959 (Complementary)

Misc_feature (2 signals)

CMV enhancer
Start: 248 End: 885
SGP(Z)
Start: 1870 End: 4288

Annotations

```
   1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
      AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

  51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
      CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

 101  TCAGGCGCGG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
      AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                          NdeI
 151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
      GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

 201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGCCCA
      GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

 251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
      AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

 301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
      AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

 351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
      TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

 401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
      TGTATTGAAT GCCATTTACC GCGCGGACCG ACTGGCGGGT TGCTGGGGGC

 451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
      GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

 501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
      GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                        NdeI
 551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
      CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

 601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
      ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                      NcoI
 651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
      TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

      NcoI
 701  GTGATCCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
      CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

 751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTCTTTT
      TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

 801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
      CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
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 851  TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
                                                 SacII
 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
                                                 SphI
1051  CCATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
1301  ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
1501  AGCGGCTCAT CGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
1601  TCGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      AGCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
1701  CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
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        RpaI
1751  TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

                                                        NcoI
1801  CGCCCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

      NcoI                                PstI        EcoRV NotI
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

      NotI XbaI
1901  CCGCTCTAGA CCAGGCGCCT GGATCGAATT GATGAAGATT AAGCCGACAG
      GGCGAGATCT GGTCCGCGGA CCTAGCTTAA CTACTTCTAA TTCGGCTGTC

1951  TGAGCGTAAT CTTCATCTCT CTTAGATTAT TTGTTTTCCA GAGTAGGGGT
      ACTCGCATTA GAAGTAGAGA GAATCTAATA AACAAAAGGT CTCATCCCCA

2001  CGTCAGGTCC TTTTCAATCG TGTAACCAAA ATAAACTCCA CTAGAAGGAT
      GCAGTCCAGG AAAAGTTAGC ACATTGGTTT TATTTGAGGT GATCTTCCTA

2051  ATTGTGGGGC AACAACACAA TGGGCGTTAC AGGAATATTG CAGTTACCTC
      TAACACCCCG TTGTTGTGTT ACCCGCAATG TCCTTATAAC GTCAATGGAG

2101  GTGATCGATT CAAGAGGACA TCATTCTTTC TTTGGGTAAT TATCCTTTTC
      CACTAGCTAA GTTCTCCTGT AGTAAGAAAG AAACCCATTA ATAGGAAAAG

2151  CAAAGAACAT TTTCCATCCC ACTTGGAGTC ATCCACAATA GCACATTACA
      GTTTCTTGTA AAAGGTAGGG TGAACCTCAG TAGGTGTTAT CGTGTAATGT

2201  GGTTAGTGAT GTCGACAAAC TAGTTTGTCG TGACAAACTG TCATCCACAA
      CCAATCACTA CAGCTGTTTG ATCAAACAGC ACTGTTTGAC AGTAGGTGTT

2251  ATCAATTGAG ATCAGTTGGA CTGRATCTCG AAGGGAATGG AGTGGCAACT
      TAGTTAACTC TAGTCAACCT GACTTAGAGC TTCCCTTACC TCACCGTTGA

2301  GACGTGCCAT CTGCAACTAA AAGATGGGGC TTCAGGTCCG GTGTCCCACC
      CTGCACGGTA GACGTTGATT TTCTACCCCG AAGTCCAGGC CACAGGGTGG

2351  AAAGGTGGTC AATTATGAAG CTGGTGAATG GGCTCAAAAC TGCTACAATC
      TTTCCACCAG TTAATACTTC GACCACTTAC CCGACTTTTG ACGATGTTAG

2401  TTGAAATCAA AAAACCTGAC GGGAGTGAGT GTCTACCAGC AGCGCCAGAC
      AACTTTAGTT TTTTGGACTG CCCTCACTCA CAGATGGTCG TCGCGGTCTG

2451  CCGATTCGGG GCTTCCCCCG GTGCCCGTAT GTGCACAAAG TATCAGGAAC
      CCCTAAGCCC CGAAGGGGGC CACGGCCATA CACGTGTTTC ATAGTCCTTG

2501  GGGACCGTGT GCCGGAGACT TTGCCTTCCA TAAAGAGGGT GCTTTCTTCC
      CCCTGGCACA CGGCCTCTGA AACGGAAGGT ATTTCTCCCA CGAAAGAAGG

2551  TGTATGATCG ACTTGCTTCC ACAGTTATCT ACCGAGGAAC GACTTTCGCT
      ACATACTAGC TGAACGAAGG TGTCAATAGA TGGCTCCTTG CTGAAAGCGA
```

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2601  CAAGGTGTCG TTGCATTTCT GATACTGCCC CAAGCTAAGA AGGACTTCTT
      GTTCCACAGC AACGTAAAGA CTATGACGGG GTTCGATTCT TCCTGAAGAA
.....
2651  CAGCTCACAC CCCTTGAGAG AGCCGGTCAA TGCAACGGAG GACCCGTCTA
      GTCGAGTGTG GGGAACTCTC TCGGCCAGTT ACGTTGCCTC CTGGGCAGAT
.....
                                  EcoRV
                                  ------
2701  GTGGCTACTA TTCTACCACA ATTAGATATC AGGCTACCGG TTTTGGAACC
      CACCGATGAT AAGATGGTGT TAATCTATAG TCCGATGGCC AAAACCTTGG
.....
2751  AATGAGACAG AGTACTTGTT CGAGGTTGAC AATTTGACCT ACGTCCAACT
      TTACTCTGTC TCATGAACAA GCTCCAACTG TTAAACTGGA TGCAGGTTGA
.....
2801  TGAATCAAGA TTCACACCAC AGTTTCTGCT CCAGCTGAAT GAGACAATAT
      ACTTAGTTCT AAGTGTGGTG TCAAAGACGA GGTCGACTTA CTCTGTTATA
.....
2851  ATACAAGTGG GAAAAGGAGC AATACCACGG GAAAACTAAT TTGGAAGGTC
      TATGTTCACC CTTTTCCTCG TTATGGTGCC CTTTTGATTA AACCTTCCAG
.....
2901  AACCCCGAAA TTGATACAAC AATCGGGGAG TGGGCCTTCT GGGAAACTAA
      TTGGGGCTTT AACTATGTTG TTAGCCCCTC ACCCGGAAGA CCCTTTGATT
.....
2951  AAAAACCTCA CTAGAAAAAT TCGCAGTGAA GAGTTGTCTT TCACAGTTGT
      TTTTTGGAGT GATCTTTTTA AGCGTCACTT CTCAACAGAA AGTGTCAACA
.....
3001  ATCAAACGGA GCCAAAAACA TCAGTGGTCA GAGTCCGGCG CGAACTTCTT
      TAGTTTGCCT CGGTTTTTGT AGTCACCAGT CTCAGGCCGC GCTTGAAGAA
.....
3051  CCGACCCAGG GACCAACACA ACAACTGAAG ACCACAAAAT CATGGCTTCA
      GGCTGGGTCC CTGGTTGTGT TGTTGACTTC TGGTGTTTTA GTACCGAAGT
.....
3101  GAAAATTCCT CTGCAATGGT TCAAGTGCAC AGTCAAGGAA GGGAAGCTGC
      CTTTTAAGGA GACGTTACCA AGTTCACGTG TCAGTTCCTT CCCTTCGACG
.....
3151  AGTGTCGCAT CTAACAACCC TTGCCACAAT CTCCACGAGT CCCCAATCCC
      TCACAGCGTA GATTGTTGGG AACGGTGTTA GAGGTGCTCA GGGGTTAGGG
.....
3201  TCACAACCAA ACCAGGTCCG GACAACAGCA CCCATAATAC ACCCGTGTAT
      AGTGTTGGTT TGGTCCAGGC CTGTTGTCGT GGGTATTATG TGGGCACATA
.....
3251  AAACTTGACA TCTCTGAGGC AACTCAAGTT GAACAACATC ACCGCAGAAC
      TTTGAACTGT AGAGACTCCG TTGAGTTCAA CTTGTTGTAG TGGCGTCTTG
.....
3301  AGACAACGAC AGCACAGCCT CCGACACTCC CTCTGCCACG ACCGCAGCCG
      TCTGTTGCTG TCGTGTCGGA GGCTGTGAGG GAGACGGTGC TGGCGTCGGC
.....
3351  GACCCCCAAA AGCAGAGAAC ACCAACACGA GCAAGAGCAC TGACTTCCTG
      CTGGGGGTTT TCGTCTCTTG TGGTTGTGCT CGTTCTCGTG ACTGAAGGAC
.....
3401  GACCCCGCCA CCACAACAAG TCCCCAAAAC CACAGCGAGA CCGCTGGCAA
      CTGGGGCGGT GGTGTTGTTC AGGCGTTTTG GTGTCGCTCT GGCGACCGTT
.....
3451  CAACAACACT CATCACCAAG ATACCGGAGA AGAGAGTGCC AGCAGCGGGA
      GTTGTTGTGA GTAGTGGTTC TATGGCCTCT TCTCTCACGG TCGTCGCCCT
.....
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3501  AGCTAGGCTT AATTACCAAT ACTATTGCTG GAGTCGCAGG ACTGATCACA
      TCGATCCGAA TTAATGGTTA TGATAACGAC CTCAGCGTCC TGACTAGTGT
.....
3551  GGCCGGAGAA GAACTCGAAG AGAAGCAATT GTCAATGCTC AACCCAAATG
      CCGCCCTCTT CTTGAGCTTC TCTTCGTTAA CAGTTACGAG TTGGGTTTAC
.....
3601  CAACCCTAAT TTACATTACT GGACTACTCA GGATGAAGGT GCTGCAATCG
      GTTGGGATTA AATGTAATGA CCTGATGAGT CCTACTTCCA CGACGTTAGC
.....
3651  GACTGGCCTG GATACCATAT TTCGGGCCAG CAGCCGAGGG AATTTACATA
      CTGACCGGAC CTATGGTATA AAGCCCGGTC GTCGGCTCCC TTAAATGTAT
.....
3701  GAGGGCTAA TGCACAATCA AGATGGTTTA ATCTGTGGGT TGAGACAGCT
      CTCCCCGATT ACGTGTTAGT TCTACCAAAT TAGACACCCA ACTCTGTCGA
.....
3751  GGCCAACGAG ACGACTCAAG CTCTTCAACT GTTCCTGAGA GCCACAACTG
      CCGGTTGCTC TGCTGAGTTC GAGAAGTTGA CAAGGACTCT CGGTGTTGAC
.....
3801  AGCTACGCAC CTTTTCAATC CTCAACCGTA AGGCAATTGA TTTCTTGCTG
      TCGATGCGTG GAAAAGTTAG GAGTTGGCAT TCCGTTAACT AAAGAACGAC
.....
3851  CAGCGATGGG GCGGCACATG CCACATTCTG GGACCGGACT GCTGTATCGA
      GTCGCTACCC CGCCGTGTAC GGTGTAAGAC CCTGGCCTGA CGACATAGCT
.....
3901  ACCACATGAT TGGACCAAGA ACATAACAGA CAAAATTGAT CAGATTATTC
      TGGTGTACTA ACCTGGTTCT TGTATTGTCT GTTTTAACTA GTCTAATAAG
.....
3951  ATGATTTTGT TGATAAAACC CTTCCGGACC AGGGGGACAA TGACAATTGG
      TACTAAAACA ACTATTTTGG GAAGGCCTGG TCCCCCTGTT ACTGTTAACC
.....
4001  TGGACAGGAT CGAGACAATG GATACCGGCA GGTATTGGAG TTACAGGCGT
      ACCTGTCCTA CCTCTGTTAC CTATGGCCGT CCATAACCTC AATGTCCGCA
.....
4051  TATAATTGCA GTTATCGCCC TATTCTGTAT ATGCAAATTT GTCTTTTAGT
      ATATTAACGT CAATAGCGAA ATAAGACATA TACGTTTAAA CAGAAAATCA
.....
4101  TTTTCTTCAG ATTGCTTCAT GGAAAAGCTC AGCCTCAAAT CAATGAAACC
      AAAAGAAGTC TAACGAAGTA CCTTTTCGAG TCGGAGTTTA GTTACTTTGG
.....
4151  AGGATTTAAT TATATGGATT ACTTGAATCT AAGATTACTT GACAAATGAT
      TCCTAAATTA ATATACCTAA TGAACTTAGA TTCTAATGAA CTGTTTACTA
.....
4201  AATATAATAC ACTGGAGCTT TAAACATAGC CAATGTGATT CTAACTCCTT
      TTATATTATG TGACCTCGAA ATTTGTATCG GTTACACTAA GATTGAGGAA
.....
4251  TAAACTCACA GTTAATCATA AACAAGGTTT GGAATTGATC TGCTGTGCCT
      ATTTGAGTGT CAATTCAGTAT TTGTTCCAAA CCTTAACTAG ACGACACGGA
.....
4301  TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCCTTGAC
      AGATCAACGG TCGGTAGACA ACAAACGCGG AGGGGGCACG GAAGGAACTG
.....
4351  CCTCGAAGGT GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG
      GGAGCTTCCA CGGTGAGGGT GACAGGAAAG GATTATTTTA CTCCTTTAAC
.....
4401  CATCGCATTG TCTGAGTAGG TGTCATTCTA TTCTGGCGGG TGCGGTGGGG
      GTAGCGTAAC AGACTCATCC ACAGTAAGAT AAGACCCCCC ACCCCACCCC
.....
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SphI

```
4451  CAGCACAGCA AGGGCGAGGA TTGGGAAGAC AATAGCAGGC ATGCTGGGGA
      GTCGTGTCGT TCCCGCTCCT AACCCTTCTG TTATCGTCCG TACGACCCCT
```

KpnI

```
4501  TGCGGTGGGC TCTATGGGTA CCCAGGTGCT GAAGAATTGA CCCGGTTCCT
      ACGCCACCCG AGATACCCAT GGGTCCACGA CTTCTTAACT GGGCCAAGGA

4551  CCTGGGCCAG AAAGAAGCAG GCACATCCCC TTCTCTGTGA CACACCCTGT
      GGACCCGGTC TTTCTTCGTC CGTGTAGGGG AAGAGACACT GTGTGGGACA

4601  CCACGCCCCT GGTTCTTAGT TCCAGCCCCA CTCATAGGAC ACTCATAGCT
      GGTGCGGGGA CCAAGAATCA AGGTCGGGGT GAGTATCCTG TGAGTATCGA

4651  CAGGAGGGCT CCGCCTTCAA TCCCACCCGC TAAAGTACTT GGAGCGGTCT
      GTCCTCCCGA GGCGGAAGTT AGGGTGGGCG ATTTCATGAA CCTCGCCAGA

4701  CTCCCTCCCT CATCAGCCCA CCAAACCAAA CCTAGCCTCC AAGAGTGGGA
      GAGGGAGGGA GTAGTCGGGT GGTTTGGTTT GGATCGGAGG TTCTCACCCT

4751  AGAAATTAAA GCAAGATAGG CTATTAAGTG CAGAGGGAGA GAAAATGCCT
      TCTTTAATTT CGTTCTATCC GATAATTCAC GTCTCCCTCT CTTTTACGGA

4801  CCAACATGTG AGGAAGTAAT GAGAGAAATC ATAGAATTTC TTCCGCTTCC
      GGTTGTACAC TCCTTCATTA CTCTCTTTAG TATCTTAAAG AAGGCGAAGG

4851  TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC
      AGCGAGTGAC TGAGCGACGC GAGCCAGCAA GCCGACGCCG CTCGCCATAG

4901  AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG
      TCGAGTGAGT TTCCGCCATT ATGCCAATAG GTGTCTTAGT CCCCTATTGC

4951  CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA
      GTCCTTTCTT GTACACTCGT TTTCCGGTCG TTTTCCGGTC CTTGGCATTT

5001  AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA
      TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG GACTGCTCGT

5051  TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT
      AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC TGTCCTGATA

5101  AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT
      TTTCTATGGT CCCCAAAGGG GGACCTTCGA GGGAGCACGC GAGAGGACAA

5151  CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG
      GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG GAAGCCCTTC

5201  CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
      GCACCGCGAA AGAGTTACGA GTGCGACATC CATAGAGTCA AGCCACATCC

5251  TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC
      AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA AGTCGGGCTG

5301  CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA
      GCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGGTTGG GCCATTCTGT
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5351  CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
      GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA TCGTCTCGCT
.....
5401  GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
      CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG ATTGATGCCG
.....
5451  TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC
      ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT TCGGTCAATG
.....
5501  CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG
      GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT TGGTGGCGAC
.....
5551  GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA
      CATCGCCACC AAAAAAACAA ACGTTCGTCG TCTAATGCGC GTCTTTTTTT
.....
5601  GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG
      CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC TGCGAGTCAC
.....
5651  GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA
      CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT AGTTTTTCCT
.....
5701  TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA
      AGAAGTGGAT CTAGGAAAAT CTAATTTTTA CTTCAAAATT TAGTTAGATT
.....
5751  AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA
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5801  GGCACCTATC TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC
      CCGTGGATAG AGTCGCTAGA CAGATAAAGC AAGTAGGTAT CAACGGACTG
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5851  TCCCCGGGGG GGGGGCGCTG AGGTCTGCCT CGTGAAGAAG GTGTTGCTGA
      AGGCCCCCCC CCCCCGCGAC TCCAGACGGA GCACTTCTTC CACAACGACT
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5901  CTCATACCAG GCCTGAATCG CCCCATCATC CAGCCAGAAA GTGAGGGAGC
      GAGTATGGTC CGGACTTAGC GGGGTAGTAG GTCGGTCTTT CACTCCCTCG
.....
5951  CACGGTTGAT GAGAGCTTTG TTGTAGGTGG ACCAGTTGGT GATTTTGAAC
      GTGCCAACTA CTCTCGAAAC AACATCCACC TGGTCAACCA CTAAAACTTG
.....
6001  TTTTGCTTTG CCACGGAACG GTCTGCGTTG TCGGGAAGAT GCGTGATCTG
      AAAACGAAAC GGTGCCTTGC CAGACGCAAC AGCCCTTCTA CGCACTAGAC
.....
6051  ATCCCTCAAC TCAGCAAAAG TTCGATTTAT TCAACAAAGC CGCCGTCCCG
      TAGGAAGTTG AGTCGTTTTC AAGCTAAATA AGTTGTTTCG GCGGCAGGGC
.....
6101  TCAAGTCAGC GTAATGCTCT GCCAGTGTTA CAACCAATTA ACCAATTCTG
      AGTTCAGTCG CATTACGAGA CGGTCACAAT GTTGGTTAAT TGGTTAAGAC
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6151  ATTAGAAAAA CTCATCGAGC ATCAAATGAA ACTGCAATTT ATTCATATCA
      TAATCTTTTT GAGTAGCTCG TAGTTTACTT TGACGTTAAA TAAGTATAGT
.....
6201  GGATTATCAA TACCATATTT TTGAAAAAGC CGTTTCTGTA ATGAAGGAGA
      CCTAATAGTT ATGGTATAAA AACTTTTTCG GCAAAGACAT TACTTCCTCT
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6251  AAACTCACCG AGGCAGTTCC ATAGGATGGC AAGATCCTGG TATCGGTCTG
      TTTGAGTGGC TCCGTCAAGG TATCCTACCG TTCTAGGACC ATAGCCAGAC
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6301  CGATTCCGAC TCGTCCAACA TCAATACAAC CTATTAATTT CCCCTCGTCA
      GCTAAGGCTG AGCAGGTTGT AGTTATGTTG GATAATTAAA GGGGAGCAGT
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6351  AAAATAAGGT TATCAAGTGA GAAATCACCA TGAGTGACGA CTGAATCCGG
      TTTTATTCCA ATAGTTCACT CTTTAGTGGT ACTCACTGCT GACTTAGGCC
.....
                  HindIII
                  ========
6401  TGAGAATGGC AAAAGCTTAT GCATTTCTTT CCAGACTTGT TCAACAGGCC
      ACTCTTACCG TTTTCGAATA CGTAAAGAAA GGTCTGAACA AGTTGTCCGG
.....
6451  AGCCATTACG CTCGTCATCA AAATCACTCG CATCAACCAA ACCGTTATTC
      TCGGTAATGC GAGCAGTAGT TTTAGTGAGC GTAGTTGGTT TGGCAATAAG
.....
                                        PvuI
                                        ====
6501  ATTCGTGATT GCGCCTGAGC GAGACGAAAT ACGCGATCGC TGTTAAAAGG
      TAAGCACTAA CGCGGACTCG CTCTGCTTTA TGCGCTAGCG ACAATTTTCC
.....
6551  ACAATTACAA ACAGGAATCG AATGCAACCG GCGCAGGAAC ACTGCCAGCG
      TGTTAATGTT TGTCCTTAGC TTACGTTGGC CGCGTCCTTG TGACGGTCGC
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6601  CATCAACAAT ATTTTCACCT GAATCAGGAT ATTCTTCTAA TACCTGGAAT
      GTAGTTGTTA TAAAAGTGGA CTTAGTCCTA TAAGAAGATT ATGGACCTTA
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6651  GCTGTTTTCC CGGGGATCGC AGTGGTGAGT AACCATGCAT CATCAGGAGT
      CGACAAAAGG GCCCCTAGCG TCACCACTCA TTGGTACGTA GTAGTCCTCA
.....
6701  ACGGATAAAA TGCTTGATGG TCGGAAGAGG CATAAATTCC GTCAGCCAGT
      TGCCTATTTT ACGAACTACC AGCCTTCTCC GTATTTAAGG CAGTCGGTCA
.....
6751  TTAGTCTGAC CATCTCATCT GTAACATCAT TGGCAACGCT ACCTTTGCCA
      AATCAGACTG GTAGAGTAGA CATTGTAGTA ACCGTTGCGA TGGAAACGGT
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6801  TGTTTCAGAA ACAACTCTGG CGCATCGGGC TTCCCATACA ATCGATAGAT
      ACAAAGTCTT TGTTGAGACC GCGTAGCCCG AAGGGTATGT TAGCTATCTA
.....
6851  TGTCGCACCT GATTGCCCGA CATTATCGCG AGCCCATTTA TACCCATATA
      ACAGCGTGGA CTAACGGGCT GTAATAGCGC TCGGGTAAAT ATGGGTATAT
.....
                                  XhoI
                                  ====
6901  AATCAGCATC CATGTTGGAA TTTAATCGCG GCCTCGAGCA AGACGTTTCC
      TTAGTCGTAG GTACAACCTT AAATTAGCGC CGGAGCTCGT TCTGCAAAGG
.....
6951  CGTTGAATAT GGCTCATAAC ACCCCTTGTA TTACTGTTTA TGTAAGCAGA
      GCAACTTATA CCGAGTATTG TGGGGAACAT AATGACAAAT ACATTCGTCT
.....
7001  CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC
      GTCAAAATAA CAAGTACTAC TATATAAAAA TAGAACACGT TACATTGTAG
.....
                  DraIII
                  ======
7051  AGAGATTTTG AGACACAACG TGGCTTTCCC CCCCCCCCCA TTATTGAAGC
      TCTCTAAAAC TCTGTGTTGC ACCGAAAGGG GGGGGGGGGT AATAACTTCG
.....
7101  ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA
      TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC TTACATAAAT
.....
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7151  CAAAAACAAA CAAATAGGGG TCCCGCGCAC ATTTCCCCGA AAAGTGCCAC
      GTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT TTTCACGGTG
.....
7201  CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG
      GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT ATTTTTATCC
.....
7251  CGTATCACGA GGCCCTTTCG TC
      GCATAGTGCT CCGGGAAAGC AG
.....
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01382

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00; C07H 21/04; C12N 15/63, 15/86, 5/10, 15/40
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450, 93.2, 93.21; 536/23.1, 23.72; 435/5, 6, 455, 457, 458, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Biosis, Medline, Biotech
Search terms: Ebola virus, glycoprotein, transmembrane, targeting

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,166,320 A (WU et al.) 24 November 1992, columns 9-10 and claims 1-18.	1-20
A	SANCHEZ et al. The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. Proceedings of the National Academy of Sciences. April 1996, Vol. 93, pages 3602-3607, especially page 3604.	3, 12
A	FELGNER et al. Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. Proceedings of the National Academy of Sciences. November 1987, Vol. 84, pages 7413-7417, especially page 7414.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier document published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search 12 APRIL 1999	Date of mailing of the international search report 11 MAY 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID GUZO Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01382

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VOLCHKOV et al. GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. Virology. 1995, Vol. 214, pages 421-430, especially page 424.	3, 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01382

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/450, 93.2, 93.21; 536/23.1, 23.72; 435/5, 6, 455, 457, 458, 320.1